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The impact of fungicide application method on soybean canopy coverage, disease, yield, seed quality, and seed fill duration.

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The impact of fungicide application method on soybean canopy coverage, disease, yield, seed quality, and seed fill duration

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ABSTRACT

As input costs continue to rise and profits fluctuate, soybean farmers have maintained interest in new fungicide applications methods. Customarily, fungicides are applied directly over the top of the soybean canopy as are herbicides and insecticides. However, fungicide application methods that could improve fungicide coverage within the canopy, such as undercover applications, has gained considerable interest. Undercover applications spray fungicides between the soybean rows, using multidirectional nozzles, and over the canopy with nozzles placed above the canopy. During 2017 and 2018, field experiments located in Iowa were used to investigate the effect of fungicide application methods on coverage, disease severity, yield, seed quality, and duration of seed fill. The objective of this research was to: (1) investigate how traditional and undercover application methods impact canopy coverage; (2) examine the effect of application method on disease control, seed quality, and yield; and (3) to determine if a fungicide application increases the rate or duration of seed fill. Studies were conducted in two small plot field experiments in 2017, six small plot field experiments in 2018, and two on-farm strip trials in 2018. Small plot and on-farm strip trials were analyzed separately.

Fungicide coverage was detected in the upper, middle, and lower canopy in two different ways: (1) water sensitive spray cards; and (2) tracer dye. Results for spray card detection in small plot experiments showed one significant difference between traditional and undercover application technologies in the upper canopy (P=0.017). The middle and lower canopies coverage values were not significant between the traditional and undercover and no canopy zone was significantly different between traditional and undercover for on-farm strip trials when using spray cards as a detection method. Conversely, the amount of tracer dye detected in the upper and lower canopy in small plot experiments differed between the traditional and undercover

(*P*=0.023 and *P*=0.034), but there were no significant differences between application method at the on-farm strip trials. For both years, the primary diseases present were frogeye leaf spot and Septoria brown spot. Greater disease severity for both diseases was observed in 2018 than in 2017. Regardless of the year, location in the canopy there was no significant difference between the treatments for foliar disease control. As a result, the inability to control disease led to there being no significant differences among seed quality and yield. Results from these trials show adequate coverage, but the inability to control disease suggests that resistance of QoI fungicide in pathogen population maybe an issue in Iowa.

The last objective of this thesis is to investigate if a QoI fungicide affects the duration of seed fill. Four small plot field experiments were conducted in 2017 and 2018. There was no significant difference in both the seed growth rate and duration of the seed filling period, but at one location there was a difference in yield. These results support other studies that suggest the probability of affecting soybean yield beyond disease control is less likely.

Soybean farmers should be aware that QoI fungicide resistance for the pathogens that cause frogeye leaf spot and Septoria brown spot have been identified in Iowa. Resistance to these two pathogens made it difficult to determine which fungicide application method was more effective. Future work comparing the traditional versus undercover applications may be necessary with more effective fungicide. Regardless of the application type it is important to only apply fungicides when necessary for disease management and to rotate mode of actions to mitigate the development of fungal resistance.

CHAPTER 1. GENERAL INTRODUCTION AND LITERATURE REVIEW

Thesis Organization

This thesis is organized into four chapters. Chapter one is the general introduction to soybean fungicide application methods. Chapter two compares the impact of two fungicide application methods on foliar coverage, disease control, yield, and seed quality in soybean. Chapter three examines how a fungicide application affects soybean seed development. Chapter four is a general conclusion.

General Introduction

Soybean (*Glycine max* [L.] Merr.) is prized for its variety of commercial uses and consistent high price value, making it one of the most valuable crops grown in the United States (Hershman et al., 2011). Since the early 2000s, fungicide applications on soybean hectares to manage soybean fungal diseases and protect yield have steadily increased (Hershman et al., 2011).

Foliar disease can be found on soybean each year, but severity differs annually, partially depending on environmental conditions (Hartman et al., 2016) The most common foliar soybean diseases in the northern United States include Septoria brown spot (*Septoria glycines*), frogeye leaf spot (*Cercospora sojina*), and Cercospora leaf blight (*Cercospora* spp.) (Hartman et al., 2016). Frogeye leaf spot and Cercospora leaf blight have the most potential to reduce yield, but most years these diseases only cause minor losses in the Midwest region of the United States (Koenning and Wrather, 2010). Management of foliar diseases often depends on resistant varieties, cultural practices, and foliar fungicides (Mueller et al., 2016). However, resistant varieties are sometimes unavailable or have lower yield potential than more susceptible varieties, and cultural practices are not feasible to implement when disease management is needed during soybean reproductive growth stages.

Foliar disease appearing during reproductive stages of soybean development can be managed using fungicide. When applying a fungicide, several factors must be considered. For example, timing of application is important since fungicides are usually more effective when applied before the onset of disease development (Hershman et al., 2011). For this reason, foliar fungicide applications on soybean in Iowa are most commonly applied at either the flowering (R1) or pod formation (R3) growth stages. Additionally, farmers must consider application rate, fungicide mode of action, the type of equipment needed to make an application, and the economics associated with making an application. In general, the best return on investment for fungicides comes when risk of important foliar diseases is sufficiently high (Hershman et al., 2011).

Since the early 2000s, fungicide application on soybean has steadily increased to manage soybean fungal disease and protect yield. Foliar fungicides also are reported to increase plant health in the absence of obvious disease (Petit el al., 2012). While the use of fungicides has steadily increased, application technique has remained relatively unchanged (Ozkan, 2016). Fungicides primarily have been applied with traditional over-the-top ground sprayers (Ozkan, 2016). The ability to use the same equipment to apply similar chemical products such as herbicide is advantageous to farmers. However, applying fungicide to a dense soybean canopy during reproductive stages can be challenging (Ozkan, 2016). The ability of fungicides to penetrate into the canopy can be hindered when using a traditional over-the-top ground sprayer (Ozkan, 2016). As a result, interest in undercover sprayers (360 Yield Center, Morton, Illinois) that may improve fungicide coverage within the soybean canopy has increased. Undercover sprayers deliver inner-canopy coverage via multi-directional spray nozzles in combination with traditional downward spray nozzles. The multi-directional spray nozzles are thought to provide enhanced fungicide coverage to the upper and lower leaves within the canopy simultaneously, but this has not been thoroughly tested.

The objective of this study is to compare undercover foliar fungicide application to traditional over-the-top application by ground sprayers and evaluate differences in coverage, foliar disease severity, seed diseases, and yield in soybean. Chapter three looks at the impact of a fungicide application from a plant health benefit. To address these objectives, two years of small plot and on-farm trials across Iowa were conducted.

Literature Review

Introduction. The number of hectares planted to soybean has increased greatly over the last several decades in the United States. In 1987, only 23.5 million hectares were planted, compared to 33.7 million hectares in 2016, an increase of more than 40 percent ("SoyStats®", 2018). The value of soybean has been one of the largest factors for the increase in hectares planted. To ensure soybean hectares remain prosperous; it will be crucial for farmers to use the best management practices, including selecting the most effective fungicide application method for management of foliar diseases of soybean.

Currently, there are several different technologies a farmer can use to make a fungicide application. It is important to understand how different equipment influences canopy coverage, foliar and seedborne diseases, yield, and plant health of soybean. Additionally, assessing how different application equipment might affect diseases that develop in the lower canopy, like Sclerotinia stem rot (*Sclerotinia sclerotiorum*), could provide insight on potential management tactics.

Fungicide Usage. The use and interest of foliar fungicides in soybean has steadily increased since the early 2000s (Wise and Mueller, 2011). Increase in fungicide use can be attributed to good efficacy against common foliar diseases, the concern over soybean diseases such as soybean rust (*Phakopsora pachyrhizi*), and for nonfungicidal plant physiological benefits (Mueller et al., 2013). Applications made to increase plant health have increased due to claims of greater chlorophyll retention, increased water and nitrogen use efficiency, and delayed senescence (Glaab and Kaiser, 1999; Grossmann and Retzlaff, 1999; Kohle et al., 2002).

Soybean Diseases. Soybean in the northern United States and Canada is most commonly impacted by Septoria brown spot (*Septoria glycines*), frogeye leaf spot (*Cercospora sojina*), Cercospora leaf blight (*Cercospora* spp.), and soybean cyst nematode (*Heterodera glycines*) Cruz et al., 2010; Dorrance et al., 2010; Hartman, 2015; Wise and Newman, 2015). The estimated economic loss due to soybean diseases from 2010 to 2014 was $26 billion in the United States and Ontario, Canada (Allen et al., 2017). Most years in Iowa, there is not enough foliar disease severity to cause yield loss, but frogeye leaf spot and Cercospora leaf blight can become yield limiting if present in high enough levels (Allen et al., 2017; Kandel et al., 2016).

Application Technology. Farmers are more accustomed to applying herbicides and insecticides to row crops than making fungicide applications (Ozkan, 2016). As a result, many farmers have existing spraying equipment to apply herbicides and insecticides over the top of the crop canopy (Ozkan, 2016).

Fungicide application technology has remained relatively unchanged, despite an increase in usage (Ozkan, 2016). Application techniques have focused primarily on nozzle selection and application timing, however; different application methods have been investigated over the years (Derksen et al., 2006b; Hanna et al., 2007). Air-assisted sprayers, canopy openers, dropleg extension pipes, and advanced sensors have been the most common fungicide application methods tested over the years (Table 1) (Derksen et al., 2006b; Hanna et al., 2007; Ozkan et al., 2006; Wolf and Daggupati, 2009; Zhu et al., 2008a,b). Recently, 360 Undercover technology (undercovers) has been marketed to improve foliar coverage for fungicide application in field crops. The interest in undercovers has been generated by the ability to deliver fungicide deeper into the crop canopy and theoretically improve coverage and product performance. It is unknown if the undercover system is superior over existing methods.

Coverage. Determining how a fungicide is distributed on a plant has primarily been accomplished using chemical analysis, visual assessment, and the use of colorimetric and fluorometric tracer dyes (Hoffmann et al., 2014). Chemical analysis is often expensive, time consuming, and hazardous (Matthews et al., 2014). Visual assessment via water sensitive cards is the most common method used to determine the relative difference in coverage for spray application methods (Panneton, 2002). However, water sensitive cards can be difficult to use due to the potential of accidental contamination via fingerprints and humidity (Cooke and Hislop, 1993). Tracer dyes have been widely used because they have no phytotoxicity to treated plants, low mammalian toxicity, and are generally inexpensive (Hoffmann et al., 2014).

Coverage – Air Assisted. Air assisted sprayers have gained interest due to their ability to place fungicide on more of the plant (Reichard et al., 1979). Investigators at Ohio State University and USDA analyzed the impact of air-assisted sprayers and coverage in the soybean canopy. Spray coverage percentage was measured in the middle canopy (60 cm from soil) and lower canopy (30 cm from soil). This study tested an air-assisted sprayer, a sprayer with canopy opener, and several traditional sprayer booms with different nozzles. Coverage was detected by water sensitive paper. The data collected showed better coverage of both the middle and lower canopy when using the air-assisted sprayer compared to a traditional sprayer (Derksen et al., 2006a).

The ability of air-assisted sprayers to provide an increase in spray coverage inside the soybean canopy has been documented (Fox et al., 2008). However, soybean farmers have been hesitant to adopt air-assisted sprayer technologies, mostly because this specialized equipment adds to the cost of application. Additionally, maintenance and installation costs are more than traditional sprayers (Fox et al., 2008). For these reasons, the air-assisted sprayer has remained a tool of higher value crops in orchards and vineyards (Fox et al., 2008).

Coverage – Electrostatic Sprayer. Electrostatic sprayers have been used in agriculture since the 1930s (Edward, 2001), and cause spray droplets to be either positively or negatively charged depending on the type of current. The advantage of this system is that charged droplets are attracted to the plant surface, which has a neutral charge. As a result, droplets land on the plant quicker, decreasing the likelihood of volatilization (Matthews, 1989). Additionally, droplets repel each other and spread out uniformly.

Electrostatic sprayers are capable of providing uniform coverage to the top of a soybean canopy. However, the electrostatic sprayers lack the ability to penetrate and reach soybean leaves within the canopy. Additionally, electrostatic sprayers have shown inconsistent results in field environments as compared to greenhouses and spray chambers (Hislop, 1988). These issues, along with the price to outfit an electrostatic sprayer, have kept them from being widely used in soybean.

Coverage – Canopy Opener. In response to expensive application technologies, focus shifted to mechanical canopy openers. Canopy openers improve coverage within the canopy while minimizing the cost of purchase, usage, and maintenance (Zhu et al., 2008). Canopy openers typically function as an adjustable metal bar that attaches to a spray boom and is capable of bending the tops of soybean plants and helping deliver fungicides deeper into the crop canopy. Canopy openers are designed in a variety of ways, but the basics include an opening device that is easily integrated onto a farmer's equipment (Zhu et al., 2008).

Investigation of canopy openers in relation to fungicide delivery were examined by using water sensitive cards. Cards were placed 30 and 60 centimeters from the ground to represent different levels within the soybean canopy. Spray coverage percentages were 5% and 4% greater in both the middle and lower canopy, respectively when using a canopy opener over a traditional ground sprayer boom (Zhu et al., 2008). The advantage of integrating canopy openers is the ability for farmers to cheaply and quickly attach a canopy opener system to their existing equipment (Zhu et al., 2008).

Coverage – Dropleg Extension Pipes. Newer technology has focused on effective and relatively inexpensive sprayer attachments. Dropleg extension pipes provides coverage to the lower surface of foliage in vegetable and field crops (Basil, 2001). Dropleg extension pipes utilize drag hoses with one or several nozzles attached at the ends (Rueegg et al., 2006).

Coverage in the canopy is increased compared to traditional sprayers when using a dropleg method due to the distribution of the spray in the canopy, which allows fungicide placement on difficult to reach plant tissues such as lower side of leaves (Rüegg and Total, 2013). Field trials out of Germany and Switzerland have shown an increase from 40% to 80% in fungicide efficacy against *Sclerotinia* spp. in French beans (*Phaseolus vulgaris*) when using dropleg extension pipes (Rüegg and Total, 2013). The combination of low cost, simple maintenance, and easy adaption to current equipment have made dropleg extension pipes popular in numerous crop species.

Coverage – 360 Undercovers. 360 Undercovers (undercovers) are designed similar to dropleg extension pipes. The undercover application is a multi-directional four-nozzle system that provides coverage within a crop canopy as well as above the canopy like a traditional sprayer. One nozzle applies product top down from a horizontal wet boom, while three other nozzles spray runs between the row to spray the sides and underside of leaves. The interest in undercover application has been on the perceived ability to provide an increase coverage that will improve product efficacy. The research and data on the increased coverage and overall effectiveness of the undercovers on soybean is limited.

Coverage - Advanced Sensors. Normally, fungicides are applied uniformly across an entire field, regardless of disease differences within the field. The combination of prescription mapping and GPS technology allows for automatic shut off of certain booms and a decrease in the volume of product used to deliver fungicide product only where needed (Seelan et al., 2003). Advanced sensors have been more commonly implemented on air-assisted sprayers and crops with a higher market value (Tackenberg et al., 2018).

Much of the research conducted on advanced sensors has been on high value tree crops and cereal grains such as wheat. Advanced sensors have been implemented on row crops for variable rate nitrogen and some herbicide applications (Tackenberg et al., 2018). However, advanced sensors for variable rate fungicides have not been conducted on soybean (Tackenberg et al., 2018). Advanced sensors have not seen adaptation to soybean due to their high cost and low potential for profitability (Zhu et al., 2008). The adoption of more expensive sprayers may increase if coupled with advanced sensors to mitigate the cost.

Coverage and Fungicide Efficacy. Beyond coverage, it is important to consider how an application technique may influence other agronomic factors. Improving fungicide coverage may have the potential to better manage seed diseases and challenging diseases like Sclerotinia stem rot that are more prominent within the soybean canopy where fungicides can be difficult to target. Examining if a certain application method impacts disease suppression and yield response may explain the benefits that a fungicide application could have.

Seed Diseases. Soybean seeds can be impacted by over thirty fungal seedborne pathogens that affect viability, germination, and seedling vigor (McGee and Nyvall, 2014). The ability of fungal pathogens to cause poorly germinating soybean seeds can be a pressing concern, especially in years with increased rainfall in the fall. Adverse weather, improper storage, insect damage, and physiological aging can form openings for fungi to enter and thus fungicide inhibit seed fungi from entering these openings has increased (Mueller et al., 2016). With an increase in soybean hectares and the introduction of new varieties, the management of fungal pathogen in soybean seed lots has become increasingly important. Two of the more common fungal seed diseases are purple seed stain and Phomopsis seed decay**.**

Purple Seed Stain. *Cercospora* spp. (Murakishi, 1951) causes Cercospora leaf blight and has the potential to cause purple seed staining wherever soybeans are grown. Some varieties can have up to 50% purple-seed stain, although trace amounts are more typical. The presence of soybean residue along with high temperature and humidity favor disease development (Jones, 1968; Schuh, 1999). Although purple seed stain does not affect yield, it can decrease seed quality that can result in product rejection or docking at market (Lehman, 1950). One widespread control method is fungicide application at the time of pod development (Grau et al., 2004) whereas cultural practices such as adopting new tillage methods are less common (Almeida et al., 2001).

Phomopsis seed decay. Phomopsis seed decay is caused by species in the *Diaporthe* genus (Kulik and Sinclair, 1999). *Diaporthe longicolla* (teleomorph) is also known as *Phomopsis* *longicolla* (anamorph) since only the conidia are observed. However, multiple species of *Diaporthe* cause soybean seed decay and this disease is now known as a complex of *Diaporthe* species. The disease is present throughout the United States and Canada and most common on susceptible varieties with excessive rain during the reproductive stages (Kulik and Sinclair, 1999; Wrather et al., 2010). Infected soybean seeds are cracked and shriveled with a white fungal growth on the surface. Additionally, infected seeds can have reduced viability, germination, emergence, and alteration of protein and oil quality (McGee. 1992; Meriles et al., 2004). Soybean seeds with high incidence of *Diaporthe* can be treated with a fungicide prior to planting to improve seed germination (Tekrony et al., 1985; Wrather et al., 2004). Seed quality response to foliar fungicide has been variable, but a reduction in infection has been reported when applications are made around the R5 growth stage (Tekrony et al., 1985)

White Mold Overview. Sclerotinia stem rot, (*Sclerotinia sclerotiorum* (Lib.) de Bary) is prominent in the northern United States and Canada (Hoffman et al., 2002). Yield can decrease by up to 2-5 bushels per hectare for every 10% increase in white mold when soybeans are at the R7 growth stage (Chun et al., 1987; Hoffman et al., 1998; Yang et al., 1999). Soybean yields and seed quality from white mold infection decrease in years of cool, wet weather (Hoffman et al., 1998; Kim et al., 1999; Wegulo et al., 1998; Yang et al., 1999). White mold is not problematic in certain years; however, it can become a significant problem in high-yielding environments and fields that have previously had Sclerotinia stem rot (Peltier et al., 2012). Since white mold is closely associated with weather and field history, future management of Sclerotinia stem rot could rely on predictive models, monitoring, and recordkeeping (Willbur et al., 2018).

White Mold Biology & Disease Cycle. White mold overwinters as hard, black sclerotia. Sclerotia in the top two inches of the soil profile germinate when soils are cool and moist (40-

60°F/5-16°C) (Adams and Ayers 1979, Grau and Hartman 1999, Wu and Subbarao 2008) to produce cup-shaped apothecia, 2 to 5 mm in diameter (Abawi and Grogan, 1979). Apothecia then produce large numbers of wind-borne ascospores capable of infecting flowers (Saharan and Mehta, 2008). Infection can progress from the flowers to the stem near a node (Grau and Hartman 1999). Subsequently, white mycelial masses and bleached lesions along the stem are the first noticeable signs. Plant lodging and wilting will lead to poor or even unfilled pods with high disease severity (Peltier et al., 2012). Finally, sclerotia develop on the outside and within the stem and pod and become dispersed by dropping to the ground or being collected with the seed during harvest (Peltier et al., 2012). White mold is most destructive when the environment is cool (lower than 85°F/30°C) and rain, fog, or dew is present with high humidity (Workneh and Yang, 2000). Additionally, soybean plants in narrow rows are at a higher risk for Sclerotinia stem rot developing (Grau and Hartman 1999).

White Mold Management. There is no completely effective management when white mold becomes problematic (Mueller et al. 2002a, 2004). However, there are numerous cultural practices, cultivar selection, and biological and chemical control practices to decrease the losses caused by white mold. For example, crop rotation with two to three years away from a host crop (Gracia-Garza et al. 2002, Rousseau et al. 2007); tillage to bury sclerotia deeper into the soil profile to decrease germination (Heffer Link and Johnson, 2007). Decreasing planting populations is also effective, since dense canopies have been shown to increase Sclerotinia stem rot incidence (Kurle et al. 2001, Lee et al. 2005). Additionally, increased row spacing (Grau and Radke 1984), altered planting date, maturity selection (Kim and Diers 2000), fertility considerations (Wallace et al. 1990, Schmidt et al. 2001), weed control (Boland and Hall 1994), and irrigation schedule (Grau and Radke 1984; Pedersen 2004) are cultural practices that may limit white mold severity. Although there are no soybean varieties with complete resistance, partial resistance is available (Grau et al. 1982, Boland and Hall 1987, Kim and Diers 2000). However, partially resistant varieties can succumb to disease if every condition of the disease triangle is adequately met (Peltier et al., 2012).

Chemical and biological control are additional approaches farmers can consider. Herbicides that contain the active ingredient lactofen may suppress Sclerotinia stem rot (Peltier et al., 2012; Wilbur et al. 2019). Lactofen can reduce Sclerotinia stem rot incidence by decreasing the soybean canopy density, which increases airflow within the canopy for a less conducive environment (Nelson et al., 2002a). Also, systemic acquired resistance can also be achieved by using lactofen. Soybean plants produce antimicrobial compounds, such as phytoalexins, to prohibit *S. sclerotiorum* growth (Dann et al., 1999; Nelson et al., 2002a,b; Landini et al., 2003). Precautions to not cause plant damage and yield loss must be taken when using herbicides in attempts to control *S. sclerotiorum.* Biological control includes using a fungus (e.g., *Coniothyrium minitans*) and incorporating it during spring before planting or possibly the previous fall (Peltier et al., 2012).

There are several different fungicide classes registered for Sclerotinia stem rot control in soybean (e.g. methyl benzimidazole carbamate, succinate dehydrogenase inhibitor, and demethylation inhibitor) (Peltier et al., 2012). These fungicide classes have limited effectiveness as they lack the ability to move downward (Mueller et al., 2013). Foliar fungicides require adequate coverage further down in the canopy where Sclerotinia stem rot infection begins. To reach within the canopy, flat-fan nozzles that produce high-fine to medium droplets are recommended (Ozkan et al., 2007). The best control can be achieved when double applications are made during the flowering period. Single applications at the beginning of flowering (R1) or full flowering (R2) result in more disease control than applications at the beginning of pod development (R3) or later (Willbur et al., 2019).

New management tools like the Sporecaster app, developed by the University of Wisconsin-Madison, can be used to determine the risk of disease development and if a foliar fungicide is necessary (Willbur et al., 2018). The most successful management programs use multiple practices with the combination of recordkeeping to track potentially problematic fields.

Soybean Plant Health. The ability of certain fungicide chemistries, like quinone outside inhibitors (QoI), to have an impact on soybean plant health in the absence of disease may be part of the steady increase of fungicide applications (Wise and Mueller, 2011). QoI fungicides are a part of the Group 11 FRAC code. Group 11 fungicides have single mode of action and include pyraclostrobin, azoxystrobin, and trifloxystrobin (Mueller et al., 2013). QoI fungicides obstruct the mitochondrial electron transfer to disrupt the synthesis of ATP (Leroux, 1996). The interest in QoI fungicide has increased due to an occurrence called the greening effect (Balba, 2007; Bartlett et al., 2002). The basic concept of the greening effect is delaying senescence and increasing the amount of time leaves remain green **(**Balba, 2007). Increasing the amount of time leaves remain green increases photosynthetic capability to increase dry matter accumulation, and theoretically increase yield (Balba, 2007; Bartlett et al., 2002). Studies of soybean physiological advantages in correlation with fungicide applications have been inconsistent (Bradley and Sweets 2008; Kyverga et al., 2013). Hormone changes may be a part of the greening effect, but the specific pathway or mechanism is not well understood (Balba, 2007).

Summary. Fungicide application equipment has changed primarily to improve coverage on crops. Today’s soybean farmers have two prevalent ground application methods options – traditional and undercover sprayers. Understanding how traditional versus undercover can differ in terms of coverage, disease control, and yield is important. Additionally, understanding how either method can impact seedborne diseases, soybean plant health, and specific diseases that develop deep in the soybean canopy, like Sclerotinia stem rot, and subsequent yield responses, will be advantageous.

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Table

Table 1. Overview of fungicide application methods to soybean.

	Efficiency (hectares per 12 hr. day)	Adaptability	Water consumption (L/Ha)	Nozzles and pressures	Cost	Success in soybean
Air-assisted Uses air to open the canopy and drive droplets deeper into the soybean canopy.	375. Limited by the maximum boom width of 18m.	Requires a tractor to either pull or haul the sprayer.	90 – 100. Water consumption is low due to small droplets and the use of air to drive droplets downward into the crop canopy.	A variety of nozzles can be used successfully depending on the desired droplet size. Pressure during application is often 275 kPa.	Average cost varies considerably, depending if sprayer is pulled or attached directly to the tractor, boom size, and tank size.	There is a concern for drift and inadequate coverage when the plant canopy is small. When applied to soybean in reproductive stages this application can achieve good coverage.

Table 1. (continued)

Electrostatic Uses a supercharger to charge the droplet so that it sticks to the plant surface.	750 depending on the boom width.	Electrostatic nozzles can be quickly attached to an existing sprayer.	140 – 281	Nozzles selection can be limited (hollow cone, air-shear) due to specificity of this system. 200 – 250 kPa is the average pressure used.	Initial set is significant at $330 per nozzle.	Allows application rate reduction while still obtaining adequate coverage. However, nozzle prices have deterred its use in soybean.
Canopy-opener Uses a bar to bend the top of the soybean plant over and as this opens the canopy, the lower leaves are sprayed.	350-750 depending on the sprayers boom width.	Designed, developed, and implemented using materials that are readily available.	140 – 281	All nozzles can be used. Pressure is similar to a traditional ground sprayer. 400 kPa is the average pressure used.	Set up costs varying but are often under $1,000.	This method delivers fungicide to the lower canopy leaves, but plant damage can also occur. Not practical for large sprayers.

Table 1. (continued)

Dropleg extension pipes Use a metal bar with one nozzle on the end that runs between the rows to spray the leaf underside and lower leaves in the canopy.	450 depending on the sprayers boom width.	Attaches to an existing sprayer boom and may require some reconfiguration.	140 – 281	A variety of nozzles can be utilized. Pressure is 250 – 300 kPa.	$7,000 to outfit 24 rows.	This method has had little testing in soybean and has been used for crops with a small plant canopy.
Undercovers Use a multidirectional nozzle body with three nozzles that run between the row to spray the underside and lower leaves. The boom also sprays downward onto the top of the crop canopy.	500 when outfitted to spray 24 rows.	This system requires a high clearance sprayer, but can be easily adapted to a spray boom.	187 – 281	A variety of nozzles can be used depending on desired droplet size. Pressure requirements can be between 400 – 450 kPa	$15,000 to outfit 24 rows with this technology.	This is the newest method available.

Table 1. (continued)

Traditional Uses boom to spray downward onto the top of the crop canopy.	750 depending on the sprayers boom width.	Requires a pull type or self-propelled sprayer often used for herbicide and insecticide applications.	140 – 281	A variety of nozzles can be used depending on the desired droplet size. Pressure requirements can be between 400 – 450 kPa.	The cost will vary tremendously depending on type (pull vs self-propelled) and overall boom width.	Most popular and widely used due to familiarity with method and success with other pesticides.
Advanced Sensors Use a camera and computer to scan ahead of a traditional sprayer boom, adjusting application rate and pressure depending on the level of disease or density in the crop canopy.	40 - 350 depending on the type of sprayer (drone vs traditional sprayer outfitted with advanced sensors).	This method requires the addition of high end cameras and a computer system to spray equipment.	Spot Spray or 94 – 281	A variety of nozzles can be used depending on the desired droplet. One advantage is differential nozzles and pressure use according to observed crop conditions.	Cost will vary. Outfitting drones is the least expensive option at $5000, and advanced sensors added to sprayers is $15,000 to $20,000.	Little testing in soybean, but more promising as cost of computer and sensors decrease.

CHAPTER 2. EFFECT OF FUNGICIDE APPLICATION METHOD ON COVERAGE, DISEASE, YIELD, AND SEED QUALITY IN SOYBEAN

Abstract

Fungicide application to soybean is a relatively new practice that became popular in the 2000s. Farmers often use top down traditional boom sprayers to apply fungicide due to availability and their experience applying herbicides and insecticides. However, it is unknown if this method provides the thorough coverage recommended on most fungicide labels. Undercover application may improve coverage using multi-directional nozzles that are placed between soybean rows in addition to top down coverage. The objective of the first study was to compare coverage between the traditional and undercover application technologies using two methods to detect coverage, spray cards and tracer dye. The second objective was to examine if application technologies differed in disease control, yield protection and seed quality preservation. Spray card indicated that both application technologies deposited 50% of the fungicide in the middle canopy. On the other hand, tracer dye detection for both application technologies had a decreased level of detection when moving from the upper to lower canopy. Disease control, yield, and seed quality did not significantly differ between methods, however pathogen resistance to the fungicide product used in the experiment may have confounded these data.

Introduction

Fungicides have become a vital crop protection product for soybean farmers. In addition to disease control, they preserve crop productivity by minimizing minor diseases on leaves that reduce photosynthesis, and enhance seed storage (McGrath, 2004). While fungicide application in soybean has increased from 11% in 2011 to 15% in 2018 (USDA NASS, 2013; USDA NASS, 2019), the fungicide application equipment has remained relatively unchanged even with the availability of new technologies. Customarily, many farmers use traditional boom sprayers that apply a fungicide downward onto a targeted surface. Many farmers use this technology for fungicides because they use the same equipment for applying herbicide and insecticide products (Ozkan, 2016). However, applying fungicide to a dense soybean canopy during its reproductive stages can present challenges. Fungicide penetration into a dense soybean canopy can be difficult when using a traditional over-the-top ground sprayer. Despite this challenge, many fungicide labels state that thorough coverage must be achieved to obtain optimal disease control. As a result, many different technologies have been developed to improve canopy penetration including: air-assisted sprayers, electrostatic sprayer, canopy openers, dropleg extensions, and advanced sensors (Derksen et al., 2006; Hanna et al., 2007; Ozkan et al., 2006; Wolf and Daggupati, 2009; Zhu et al., 2008a,b). Only a few of these have garnered as much attention as the undercover fungicide application (360 YieldCenter, Morton, Illinois) because of its potential to increase coverage using multi-directional nozzles above and between the crop rows.

Visual assessment, colorimetric and fluorometric tracer dyes, and chemical analysis have been used to detect pesticide spray coverage (Hoffmann et al., 2014; Cooke and Hislop, 1993; Cai and Stark, 1997; Pergher, 2001). Visual assessment is a relatively quick method, but is prone to inconsistent readings due to human error if a calibrated program isn't used to read the value (Cooke and Hislop, 1993). Colorimetric and fluorometric tracer dyes are inexpensive and have low toxicity to plants and mammals, but suffer light and heat degradation (Cai and Stark, 1997; Hoffmann et al., 2014; Pergher, 2001). Chemical analysis provides accurate results, but can be hazardous to work with in field conditions (Pergher, 2001).

Significant yield loss or affected seed quality is attributed to a pathogen's ability to cause infections at different locations within the plant canopy (Gimenes et al., 2013). Thus, farmers may decide to use fungicides to prevent yield losses and preserve soybean quality from fungal pathogens. With increases in application costs (Edwards et al., 2011), improving coverage may increase the value of a fungicide application by improving disease control and ultimately protecting more yield. The objective of this study was to compare canopy coverage of the traditional and undercover fungicide application technologies and evaluate how canopy coverage effects disease control, yield, and seed quality under a range of field conditions.

Materials and Methods

Field studies were conducted in two locations in 2017 and six locations in 2018 in Iowa, for a total of eight site-years (Table 1). Of these, six were small plots, two in 2017 and four in 2018; and two were on-farm trials in 2018. All locations included three treatments: (1) non-treated control, (2) fungicide applied using a traditional sprayer, and (3) fungicide applied using an undercover sprayer. Small-plot fields were laid out as a randomized complete block design (RCBD) with eight replications. Each plot replication consisted of four rows (6.1 m long with 76.2 cm row-row spacing). The design of the on-farm trials was also a RCBD with three replications. Each plot was 1.2 km long with 76.2 cm row-row spacing. Weeds and insects were managed according to local recommendation (Table 1).

Fluxapyroxad and pyraclostrobin (Priaxor, BASF, Ludwigshafen, Germany) was applied at 292 mL per hectare at the R3 growth stage (beginning pod, Fehr and Caviness, 1977). At all locations, the soybean canopy was closed at the time of application. For small plots, the traditional sprayer was a self-propelled research sprayer built by Iowa State University personnel. The traditional sprayer had an over-the-canopy boom equipped with XR11003 nozzles (TeeJet, Glendale Heights, Illinois) and the undercover sprayer, which was plumbed to the self-propelled research sprayer built by Iowa State University personnel and had XR11002 nozzles (TeeJet). The same nozzles were used for both small plot and on-farm trials based on desired application rate. Small plot traditional applications were made at a speed of 6.4 km/h, with 207 kPa to deliver 187 L/ha. Small plot undercover applications were made a speed of 6.4 km/h, with 276 kPa, to deliver 187 L/ha. On-farm plots were sprayed with a Hagie STS 12 (Hagie Manufacturing Company, Clarion, Iowa) that traveled at a speed of 8 km/h with 345 kPa to deliver 187 L/ha for the traditional application and 11.2 km/h with 414 kPa to deliver 187 L/ha for the undercover application.

Fungicide coverage. Two detection methods were used to determine fungicide coverage within the soybean canopy; (1) water sensitive spray cards (Spraying Systems Co., Wheaton, Illinois) and (2) 1,3,6,8-Pyrenetetrasulfonic acid tetrasodium salt (Spectra Color Corp, Kearny, New Jersey) dye. Samples for both methods were collected from three canopy zones: 1) the lower canopy was denoted as the lowest six nodes on the soybean plant; 2) the middle canopy was the bulk of the soybean plant, usually consisting of 10 center nodes; and 3) the upper canopy was represented by the top 2 to 3 nodes of the soybean plant.

Visual assessments were conducted with water sensitive spray cards that measured 52 x 76 mm (Fig 1). Six cards per plant were placed on the adaxial and abaxial surface of the center leaf of the trifoliate located at the top, middle, and bottom of the soybean canopy. Following application, the spray cards were photographed and the percent of each card covered with spray droplets calculated using an image analysis system (Spray Card Analysis App., Iowa State University Extension and Outreach).

The fluorometer tracer dye method used 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt. The salt was dissolved in a spray tank at a rate of 6.1 grams of dye per 100 liters (Fritz et al., 2011). Following application one trifoliate from the top, middle, and bottom canopies (as previously described) were sampled from each replication. Leaves were placed into individually labeled plastic bags (26.8cm x 27.3cm) with forty mL of 10% isopropyl alcohol. The plastic bag was agitated by hand for approximately 15 s, and 3.5 mL of the effluent was decanted into a plastic UV-visible cuvette (10mm x 10mm x 45mm) (Azzota Corporation, Claymonet, Delaware). The cuvettes were placed into a Trilogy Laboratory Fluorometer that had a Trilogy Module: PTSA reader (Turner Designs, San Jose, California). Fluorometric readings were converted from parts per million (ppm) to milligram/liter (mg/L). Data collection for both detection methods was done in the field within thirty minutes of the application.

Spray coverage was analyzed using direct values detected and as a distribution. To determine the distribution, the spray card values from abaxial and adaxial surface of a leaf were combined. This process was done for all three canopy zones. The total value of all spray cards representing the upper, middle, and lower canopy were then added together. The value of each of the three canopy zones was divided by the total value of all three cards combined and multiplied by one hundred to get percent coverage of the fungicide in each section of the canopy. This distribution method was also used for tracer dye detection method, but no values were combined because this method does not differentiate between the abaxial and adaxial surface of the leaves.

Foliar diseases and grain yield. Foliar diseases were assessed at the R6 growth stage (full seed, Fehr and Caviness, 1977) from the center rows of each small plot and rows seven and eight for the on-farm strip trials. Ten leaves in both the upper and middle canopy were chosen arbitrarily and frogeye leaf spot severity was visually estimated as the percent leaf area blighted. Septoria brown spot (*Septoria glycines*) severity was assessed by visually estimating the percent of leaf area covered by the disease on ten arbitrary leaves at the highest point of infection within the canopy.

For the small plot trials, seed was mechanically harvested from the two center rows at the R8 (full maturity; Fehr and Caviness, 1977) growth stage with a 2009 Almaco SPC20 research plot combine (ALMACO, Nevada, Iowa). Total seed weight per plot and moisture were measured. Seed weight was adjusted to 13% moisture and yield was calculated. On-farm strip trials were harvested with calibrated John Deere S670 (John Deere, Moline, Illinois) and Case iH 9240 (Case iH, Racine, Wisconsin) combines. Harvesters were calibrated the Climate Feildview Application (Monsanto Co, St Louis, Missouri) and SMS software (Ag Leader, Ames, Iowa).

Seed quality. Each year a subsample of seed was collected from nine plots (3 replications of 3 treatments) at two of the small plot locations (2017; Roland and Story City, Iowa) (2018; Curtiss and Hinds Research Stations, Ames, Iowa). Seed subsamples were collected at harvest and tested for pathogens associated with the seeds. First, 100 seeds were selected arbitrarily from the 1361 g of seed per plot. The selected seeds were immersed in a 1% sodium hypochlorite for 30 seconds followed by a triple rinse in sterile water. Two sterile blotter papers were placed within a blotter box and moistened with 63ml of sterile water containing 0.0315g of dicloran fungicide (Botran 75W, Gowan, Canada) to prevent contamination. Seeds were placed aseptically in the blotter box that was incubated in the dark for 7 days at 25°C (McGee, 1986). Microscope identification was used to identify the fruiting bodies on infected seed and the seed infected with *Diaporthe* spp. were counted.

Data Analysis. Analysis was performed using Proc GLIMMIX (version 9.4; SAS Institute Inc., Cary, North Carolina) for coverage, disease control, grain yield, and seed quality. Data analysis was performed separately for small plot and on-farm strip trial. For each trial type, individual locations were analyzed separately and were then also pooled for combined analysis. The fungicide treatment (application method) was treated as a fixed factor and replication was treated as a random factor. Fisher's protected least significant difference (LSD) was used to separate the treatment means at an alpha (α) level of 0.05.

Results

Coverage. Both detection methods resulted in similar coverage in the lower and upper canopy for both fungicide application technologies. Spray card detection, averaged across all small plot locations and years, differed between application technology only in the middle canopy where more coverage was measured on abaxial surface of the leaf using the undercover technology (P=0.014) (Table 3). Separate analyses for each location-year showed that the undercover nozzles had greater coverage on the adaxial leaf surface in the middle canopy at all locations except in Nashua, IA where the opposite trend was observed (Table 4). No significant difference between either application technology at any of the six spray cards on the soybean plant (Table 4) was detected in the on-farm strip trials. Similarly, no significant difference in coverage in any of three canopy zones was detected using tracer dye detection for either application technology when averaged across all small plot and on-farm locations (Table 3).

Coverage Distribution. Besides looking at coverage as a direct value, coverage distribution in the upper, middle and lower canopy was calculated as a percentage of total fungicide sprayed. Analyzing coverage as a distribution (Figure 2,3) was done for both spray cards and tracer dye detection methods. The spray card method (pooled across all small plot location-years) showed no differences in spray distribution for either traditional or undercover technologies, except in in the upper canopy where the traditional application had a higher amount than the undercover application (P=0.017; Table 5). Similarly, the tracer dye detection method in the small plots (Table 5) showed more fungicide distributed in the upper canopy with the traditional application technology than the undercover application technology (P=0.023).

Conversely, in the lower canopy the undercover application had greater amounts of fungicide distributed in the upper canopy compared to the middle and lower canopy (Table 5). No significant differences in the distribution of fungicide coverage among the three canopy levels was detected on the on-farm strip trials for both spray card and tracer dye detection (Tables 5).

Disease Control and Yield. Frogeye leaf spot and Septoria brown spot were the two most common diseases observed at all the locations and disease severity was numerically greater in 2018 than 2017. Also, frogeye leaf spot severity was numerically greater in small plots than on-farm trials. Frogeye leaf spot and Septoria brown spot severity was not affected by fungicide application treatment (Table 7) regardless of location in soybean canopy in both small plots and on-farm trials.

Yield was slightly greater in small plot than in on-farm trials (Table 7). Differences in yield between application treatments were non-significant at seven of eight experimental sites, except for one location, Hinds, Ames where yield for the traditional application treatment was 7% greater than the untreated control, while yields of the undercover and untreated control were not different (P=0.001, Table 8).

Seed Quality. Seed quality differed considerably in 2017 and 2018 because of different levels of *Diaporthe* spp. (Table 9 and 10). In 2018, there was 15% increase in the average number of seeds infected with *Diaporthe* spp. than 2017, likely due to different environments during the reproductive growth stages (Table 2). There was 53% more precipitation during the month of October 2018 than October 2017. Harvesting in 2018 was also impacted due to excessive rain in 2018, which may have caused greater seed infection. However, fungicide application did not affect seed quality in both years regardless of the level of *Diaporthe* spp. (P=0.513 in 2017 and P=0.496 in 2018) (Table 9).

Discussion

To our knowledge, this is the first study comparing undercover application and traditional application and evaluating PTSA tracer dye as a detection method for fungicide applications. This research describes how incorporating tracer dye to track fungicide coverage can be effective, consistent, and time efficient. Growing interest in fungicide application methods requires greater understanding of how different application technologies can impact coverage. The use of tracer dye to evaluate coverage of a fungicide application is relatively undocumented, although it is not a new method.

Overall, both methods successfully measured fungicide coverage. However, when comparing detection methods there were slight differences. Spray card detection showed most of the fungicide application landing in the middle of the soybean canopy. Conversely, the tracer dye showed a consistent reduction in spray coverage when moving from the upper canopy to the lower canopy. The tracer dye method provided quicker results and was considerably less labor intensive than the spray card method.

In general, there is limited information regarding undercover spray application. Our results showed minimal differences in coverage between fungicide application technologies which was inconsistent from previous studies. For example, Hoffmann et al. (2019) reported that leaf surface coverage for traditional applications was 13% greater in the upper canopy than other zones in the soybean canopy.

While most fungicide labels include a statement that achieving thorough coverage will increase fungicide efficacy it is also important to consider how different fungicides move in the plant. For example, contact fungicides remain at the location on which they land. Contact fungicides cannot protect new growth and degrade with precipitation and light. As a result, achieving adequate coverage is critical when a contact fungicide is used. On the other hand, systemic fungicides are absorbed into the plant tissue. These fungicides are capable of translaminar movement allowing the fungicide to disperse on the plant surface and then move inside the plant's tissue. One extra advantage of systemic fungicides is their ability to protect the underside of leaves. Specific systemic fungicides, such as QoIs can move upward in the xylem and as a gas to rebind to the waxy leaf surface.

Although both application technologies provided coverage in the upper, middle, and lower soybean canopy zones, in most cases and we detected inadequate disease control and no differences in yield. The ineffectiveness of the fungicide to control both diseases we evaluated could be attributed to QoI fungicide resistance (Zhang et al., 2018; Neves et al., 2019). Fluxapyroxad and pyraclostrobin was one of the most frequently applied fungicide in commercial soybean, however a reduction in its efficacy has been documented (Mueller et al., 2019). In order to make a reasonable recommendation regarding the effect of application technology on disease control, additional testing of both technologies using fungicide with multiple effective chemistries against frogeye leaf spot and Septoria brown spot will be necessary. In addition, evaluation of the undercover sprayer when white mold is present could be of benefit, since this disease develops in the low to mid canopy.

Beyond foliar diseases, fungal pathogens can impact seed quality. Decreased seed quality due to *Diaporthe* spp. can result in decreased germination, emergence, and alteration in protein content (McGee, 1992; Meriles et al., 2004). Successful control of *Diaporthe* spp. by fungicides has not been consistently reported. For example, in 2009, a foliar application of the QoI fungicide pyraclostrobin at the R3 + R5 (beginning seed) growth stages significantly reduced stem and seed infection caused by *Diaporthe* spp. (Tekrony et al., 1985; Wrather et al., 2004).

However, other studies reported an application of another QoI fungicide, azoxystrobin, application at the R3 growth stage (beginning of pod) increased seed infection by *Diaporthe* spp. as compared to untreated control (Wrather et al., 2004). One potential reason for the difference in the ability of fungicide applications to control *Diaporthe* spp. is the weather during the growing season and its ability to create a favorable environment for disease development. The timing of application maybe another factor if infection occurs during the R5 growth stage.

Conclusion paragraph Fungicide application technologies have received considerable interest as they provide the potential to increase profitability as profits margins become tighter in soybean production. There was no difference between the traditional and undercover application for controlling foliar diseases and protecting yield of soybean. Fungicide resistance is an important factor when considering making an application. Future work with multiple fungicide chemistries will be necessary to better determine differences in fungicide application methods.

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Figures

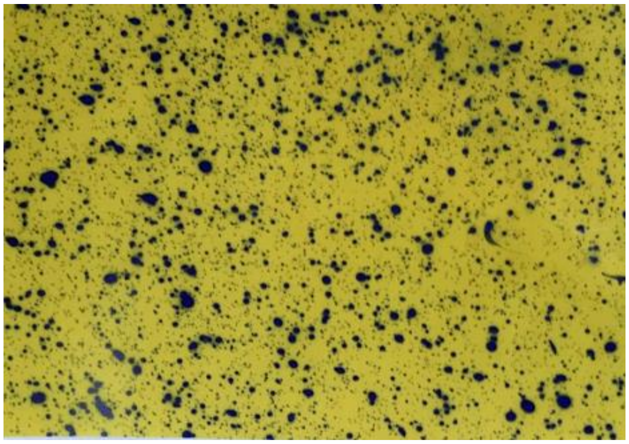

Figure 1. Water sensitive card (52 x 76mm) following application of a fungicide. Percent coverage of the card was estimated using spray card analysis application.

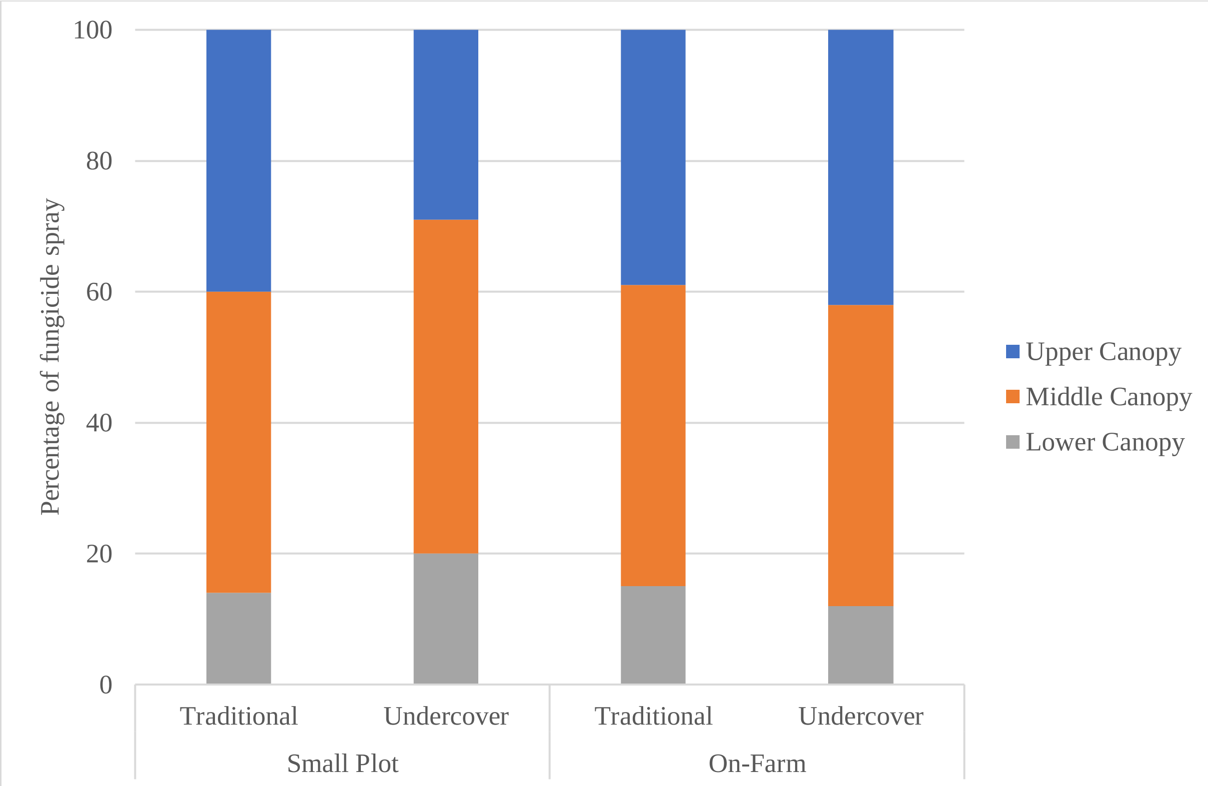

Figure 2. Distribution of fungicide applied using traditional or undercover technology to soybean as detected by spray cards. Coverage was detected using spray cards placed within the soybean canopy that was divided into thirds. The lower canopy was represented by the lowest six nodes on a soybean plant. The middle canopy was represented the bulk of a soybean plant, usually this region consisted of 10 nodes. The upper canopy was represented by the top 2 to 3 nodes of a soybean plant.

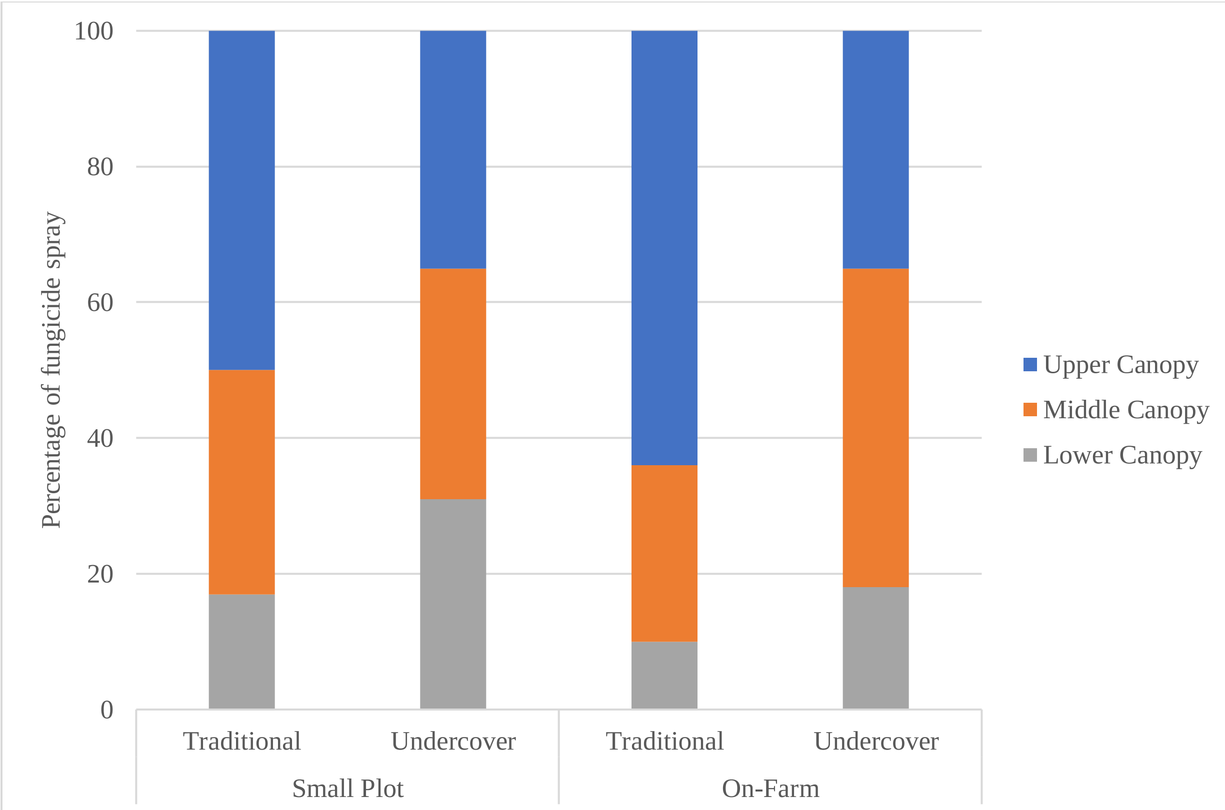

Figure 3. Distribution of fungicide applied using traditional or undercover technology to soybean as detected by tracer dye. Coverage was analyzed by dividing a soybean plant into thirds. The lower canopy was represented by the lowest six nodes on a soybean plant. The middle canopy was represented the bulk of a soybean plant, usually this region consisted of 10 nodes. The upper canopy was represented by the top 2 to 3 nodes of a soybean plant.

Tables

Table 1. Year, location, GPS coordinates, cultivar, planted population, and herbicide information for small plot and on-farm experiment sites in Iowa from 2017 to 2018, examining the impact of fungicide application method on soybean.

					Herbicide[a]	
Year	Location (Iowa)	GPS Coordinates	Cultivar	Planted population (seeds/ha)	Preemergence	Postemergence
2017	Roland	42.123552, -93.500943	S28C6	309,405	pyroxasulfone, metribuzin,	glyphosate
2017	Story City	42.202926, -93.541542	AG2535	334,158	pendimethalin, pyroxasulfone	glyphosate, imazamox
2018	Curtiss, Ames	42.003574, -93.662752	S28C6	309,405	saflufenacil	glyphosate
2018	Hinds, Ames	42.061962, -93.617633	S28C6	309,405	pendimethalin	glyphosate
2018	Kanawha	42.932093, -93.798008	S28C6	309,405	pyroxasulfone	glyphosate + clethodim
2018	Nashua	42.937892, -92.569691	S28C6	309,405	saflufenacil, metribuzin	glyphosate + clethodim
2018	Nevada	41.971845, -93.510850	AG2535	334,158	*S*-metolachlor + pendimethalin	glyphosate
2018	Roland	42.166086, -93.483307	AG2535	334,158	*S*-metolachlor + pendimethalin	glyphosate

[a] Roland, IA 2017 – (Zidua, 2.0 oz./acre, BASF), (Sencor 75DF, 150 g/acre, Bayer Environmental Science) preemergence, (RoundUp PowerMAX, 48 oz./acre, Bayer Environmental Science) postemergence. Story city, IA 2017 – (Prowl H2O, 24 oz./acre, BASF), (Zidua PRO, 4.5 fl. oz./acre, BASF) preemergence, (Touchdown total 64 fl. oz./acre, Syngenta), (Raptor, 4 fl. oz./acre, BASF) postemergence. Curtiss farm, IA 2018 – (Sharpen, 1.0 fl.oz./acre, BASF) preemergence, (RoundUp WeatherMAX, 42 fl.oz./acre, Bayer Environmental Science). Hinds farm, IA 2018 – (Prowl H2O, 48 oz./acre, BASF) preemergence, (RoundUp PowerMAX 40 fl.oz./acre Bayer Environmental Science) postemergence. Kanawha, IA 2018 - (Zidua, 2.0 oz./acre, BASF) preemergence, (Buccaneer Plus, 40 oz./acre, Tenkoz), (First Rate, 2 oz./acre, Dow AgroSciences) postemergence. Nashua, IA 2018 – (Sharpen, 1.0 fl.oz./acre, BASF), (Sencor 75DF, 150g/acre, Bayer Environmental Science) preemergence, (RoundUp WeatherMAX, 42 fl.oz./acre, Bayer Environmental Science), (First Rate, 2 oz./acre, Dow AgroSciences) postemergence. Nevada, IA 2018, (Dual II Magnum, 26 fl.oz./acre, Syngenta), (Prowl H2O, 24 oz./acre, BASF) preemergence, (RoundUp PowerMAX 40 fl.oz./acre, Bayer Environmental Science) postemergence. Roland, IA 2018 - (Dual II Magnum, 26 fl.oz./acre, Syngenta), (Prowl H2O, 24 oz./acre, BASF) preemergence, (RoundUp PowerMAX 40 fl.oz./acre, Bayer Environmental Science) postemergence.

Table 2. Monthly precipitation during the soybean growing season in Iowa from 2017 and 2018.

		Monthly precipitation (cm)[a]						
Year	Location (Iowa)[b]	May	June	July	August	September	October	Total
2017	Roland	10.1	4.3	3.6	5.1	5.1	6.5	34.7
2017	Story City	12.2	5.9	3.6	5.4	4.3	7.3	38.7
2018	Curtiss, Ames	8.7	2.9	4.1	6.4	8.6	12.4	43.1
2018	Hinds, Ames	10.1	3.8	3.2	5.7	9.6	11.4	43.8
2018	Kanawha	12.3	5.6	4.7	7.1	8.4	9.5	47.6
2018	Nashua	13.1	4.7	6.7	4.9	5.5	8.8	43.7
2018	Nevada	9.7	3.4	5.4	6.1	7.4	10.5	42.5
2018	Roland	8.5	5.4	6.1	7.2	8.8	11.6	47.6

[a] Natural precipitation.

Table 3. Percent coverage of soybean canopy detected using spray cards placed within the canopy and tracer dye (mg/L) detected Iowa in 2017 and 2018.

	Upper Canopy[a]			Middle Canopy			Lower Canopy		
	Spray cards[b]		Dye[c] (mg/L)	Spray cards		Dye	Spray cards		Dye
	Adaxial	Abaxial		Adaxial	Abaxial		Adaxial	Abaxial	
Small Plot[d]									
Traditional[e]	13.9	1.6	2.2	17.8	1.7	1.2	5.4	0.5	0.7
Undercover	12.1	3.3	1.1	20.8	10.8	0.9	12.4	0.6	0.7
P-value[f]	0.968	0.589	0.302	0.335	0.014	0.115	0.131	0.809	0.773
On-Farm									
Traditional	14.8	1.6	1.9	21.2	10.3	0.8	6.1	0	0.3
Undercover	16.2	4.4	1.2	16.3	11.4	1.6	7.3	0	0.7
P-value	0.409	0.343	0.234	0.111	0.813	0.735	0.305	-	

[a] Lower canopy was represented by the lowest six nodes on the soybean plant. Middle canopy was represented the bulk of the soybean plant, usually this region consisted of 10 nodes. Upper canopy was represented by the top 2 to 3 nodes of the soybean plant.

[b] Two spray cards were paper clipped to one leaf to represent the adaxial and abaxial of the leaf in that zone. Spray cards were analyzed for percentage of the card covered by computer analysis.

[c] Tracer dye was added to the spray tank and following application one trifoliate was removed from each of the three canopy zones. The trifoliates were placed into a plastic bag with 10% isopropyl alcohol. Trifoliates were agitated and effluent was drawn out and placed into a UV-visible cuvette. The cuvette was then placed into a triology fluorometer for a milligram/liter (mg/L) reading. There is only on reported value for each of the 3 canopy zones due to the inability to separate the adaxial and abaxial of leaves using the dye detection method.

[d] Field trials were small plot design and replicated on-farm strip. Small plots were arranged in a randomized complete block design with 8 replications at each of the 6 locations. There were 2 on-farm strip trial locations with 3 replications at each location.

[e] Traditional application applied fungicide directly over the top of a crop canopy. Undercover application applied fungicide over the top of the crop canopy and within the crop canopy using 3 multidirectional nozzles placed between the rows.

[f] Fisher's protected least significant difference (LSD) was used to separate the treatment means at an alpha (α) level of 0.05

Table 4. Percentage of spray card coverage and tracer dye detection (mg/L) on soybean of two fungicide application technologies in Iowa in 2017 and 2018.

	Upper canopy[a]			Middle canopy			Lower canopy		
	Spray cards[b]		Dye[c]	Spray cards[b]		Dye[c]	Spray cards[b]		Dye[c]
Small Plot	Adaxial	Abaxial		Adaxial	Abaxial		Adaxial	Abaxial	
Roland									
Traditional	15.7	0	1.1	16.5	0	1.9	11.9	3.1	0.3
Undercover	14.9	0	0.9	30.2	2.3	0.7	17.2	1.4	1.4
P-value[g]	0.689	-	0.238	0.067	0.062	0.009	0.296	0.591	0.017
Story City									
Traditional	7.2	0	1.2	12.1	1.1	1.6	5.5	0	0.2
Undercover	7.3	0	0.9	20.7	6.1	1.1	9.4	1.6	0.9
P-value	0.974	-	0.102	0.158	0.109	0.091	0.441	0.349	0.048
Curtiss, Ames									
Traditional	8.1	0.7	1.6	7.1	1.4	0.9	1.9	0	2.1
Undercover	3.7	1.2	0.6	7.2	2.4	0.5	1.2	0.3	0.6
P-value	0.008	0.313	0.007	0.964	0.679	0.237	0.334	0.085	0.009
Hinds, Ames									
Traditional	20.1	5.4	2.4	21.9	7.7	0.7	1.91	0	0.7
Undercover	18.1	6.9	1.5	23.2	25.4	0.7	20.4	0.1	0.6
P-value	0.774	0.822	0.357	0.781	0.074	0.787	0.04	0.351	0.879
Kanawha									
Traditional	20.2	0.9	3.9	12.1	0	1.3	3.1	0	0.4
Undercover	18	5.1	2.2	22.5	13.1	2.1	4.4	0	0.4
P-value	0.864	0.082	0.186	0.437	0.319	0.072	0.087	-	0.956
Nashua									
Traditional	13.5	1.4	1.6	36.9	1.1	0.6	7.9	0	0.3
Undercover	9.8	7.8	0.2	21.4	15.4	0.3	21.9	0	0.4
P-value	0.502	0.203	0.111	0.101	0.035	0.018	0.072	-	0.676
On-Farm									
Nevada									
Traditional	12.1	0	2.1	33.9	2.6	0.9	1.7	0	0.4
Undercover	16.7	2.5	1.6	12.4	13.8	1.6	8.1	0	0.7
P-value	0.304	0.274	0.224	0.292	0.283	0.379	0.444	-	0.321
Roland									
Traditional	12.9	0	1.8	11.1	0	0.7	10.4	0	0.2
Undercover	30.2	6.4	0.8	8.2	9.1	1.6	6.6	0	0.7
P-value	0.105	1.88	0.087	0.739	0.197	0.075	0.553	-	0.451

[a] Lower canopy was represented by the lowest six nodes on the soybean plant. Middle canopy was represented the bulk of the soybean plant, usually this region consisted of 10 nodes. Upper canopy was represented by the top 2 to 3 nodes of the soybean plant.
[b] Two spray cards were paper clipped to one leaf to represent the adaxial and abaxial of the leaf in that zone. Spray cards were analyzed for percentage of the card covered via spray card analysis application
[c] Tracer dye is presented in mg/L.
[d] All field experiments were conducted across Iowa. In 2018, there were two locations in Ames at separate research farmers.
[e] Traditional application applied fungicide directly onto the top of a crop canopy. Undercover application applied fungicide to the top of the crop canopy as well as depositing it between the

Table 4. (continued)

rows with 3 multidirectional nozzles to increase the chance of applying a fungicide to the lower and underside of leaves within the soybean canopy.

[f] Field trials were conducted in small plot in university research farm and replicated on-farm strip trial in farmer's field. Small plots were arranged in a randomized complete block design with 8 replications at each of the 6 locations. There were 2 on-farm strip trial locations with 3 replications at each location.

[g] Fisher's protected least significant difference (LSD) was used to separate the treatment means at an alpha (α) level of 0.05.

Table 5. Distribution (percentage) of fungicide in the canopy of soybean measured using spray cards and tracer dye detection.

	Small plot[a]			On-farm		
Spray Card	Traditional	Undercover	*P*-value[b]	Traditional	Undercover	*P*-value
Upper Canopy[c]	40[d]	29	0.017	39	42	0.575
Middle Canopy	46	51	0.364	46	46	0.953
Lower Canopy	14	20	0.134	15	12	0.621
Tracer Dye						
Upper Canopy	50	35	0.023	64	35	0.305
Middle Canopy	33	34	0.603	26	47	0.487
Lower Canopy	17	31	0.034	10	18	0.786

[a] Small plots were arranged in a randomized complete block design with 8 replications at each of the 6 locations. There were 2 on-farm strip trial locations with 3 replications at each location.
[b] Fisher's protected least significant difference (LSD) was used to separate the treatment means at an alpha (α) level of 0.05
[c] Lower canopy was represented by the lowest six nodes on the soybean plant. Middle canopy was represented the bulk of the soybean plant, usually this region consisted of 10 nodes. Upper canopy was represented by the top 2 to 3 nodes of the soybean plant.
[d] Distribution was calculated by adding the spray card values of the adaxial and abaxial side of a single leaf together as one value. The total value of all spray cards representing the upper, middle, and lower canopy were then added together. The value of each of the three canopy zones was divided by the total value of all three cards combined.

Table 6. Year, location, variety, seed treatment, application date at the R3 growth stage, disease assessment, and harvest date for experimental sites in Iowa from 2017 to 2018, examining the impact of fungicide application technology on disease control, seed quality, and yield.

Year	Location (Iowa)[a]	Variety	Seed Treatment[c]	Application Date[d]	Disease assessment	Harvest date
2017	Roland	S28C6	thiamethoxam, mefenoxam, fludioxonil	4-Aug	26-Aug	18-Oct
2017	Story City	AG2535	NA[e]	4-Aug	26-Aug	16-Oct
2018	Curtiss, Ames	S28C6	thiamethoxam, mefenoxam, fludioxonil	18-July	1-Sep	20-Oct
2018	Hinds, Ames	S28C6	thiamethoxam, mefenoxam, fludioxonil	18-July	1-Sep	18-Oct
2018	Kanawha	S28C6	thiamethoxam, mefenoxam, fludioxonil	25-July	8-Sep	18-Oct
2018	Nashua	S28C6	thiamethoxam, mefenoxam, fludioxonil	31-July	7-Sep	23-Oct
2018	Nevada	AG2535	clothianidin, *bacillus firmus* I-1582	1-Aug	31-Aug	22-Oct
2018	Roland	AG2535	clothianidin, *bacillus firmus* I-1582	1-Aug	3-Sep	24-Oct

[a] All field experiments were conducted across Iowa. In 2018, there were two locations in Ames at separate research farmers.
[b] SP = small plot. OFST = On-farm strip trial. Experimental field design was either small plot design or on-farm strip trial There were 6 small plot locations and all were arranged in a randomized complete block design with 8 replications at each location. There were 2 on-farm strip trial locations with 3 replications at each location.
[c] Seed treatments: thiamethoxam + mefenoxam + fludioxonil (Cruiser Maxx 0.04 mg a.i/seed), clothianidin + *Bacillus firmus* I-1582 (Poncho VOTiVO 0.13 mg a.i/seed).
[d] All applications were made at the R3 growth stage is the beginning of pod development where the pods are 5mm in size at one of the four uppermost nodes (Fehr and Caviness, 1977).
[e] NA = not available.

Table 7. Treatment (traditional, undercover, and non-treated) effect on frogeye leaf spot, Septoria brown spot, and yield in Iowa in 2017 and 2018.

		Upper canopy[a]	Middle canopy[a]		
		Frogeye leaf spot severity[b]	Frogeye leaf spot severity	Septoria brown spot severity[c]	Yield (kg/ha)[d]
Small plot[e]	Non-treated[f]	4.3	3.6	13.1	3894
	Traditional	3.9	3.3	12.6	3981
	Undercover	4.3	3.4	13.8	3887
	P-value[g]	0.481	0.121	0.309	0.752
On-farm	Non-treated	1.8	1.4	13.1	3571
	Traditional	1.8	1.4	13.1	3578
	Undercover	1.6	1.4	11.6	3517
	P-value	0.122	0.865	0.114	0.313

[a] Disease was measured in different canopy zones. Middle canopy was represented the bulk of the soybean plant, usually this region consisted of 10 nodes. Upper canopy was represented by the top 2 to 3 nodes of the soybean plant.
[b] Frogeye leaf spot severity was determined estimating the percentage of the leaf area diseased.
[c] Septoria brown spot severity was determined by selecting the uppermost leaf infected with disease and estimating the percentage of the leaf area diseased.
[d] Yields were adjusted to 13% seed moisture.
[e] Field trials were conducted in small plot in university research farm and replicated on-farm strip trial in farmer's field.
[f] Non-treated = untreated control. Traditional application applied fungicide directly onto the top of a crop canopy. Undercover application applied fungicide to the top of the crop canopy as well as depositing it between the rows with 3 multidirectional nozzles to increase the chance of applying a fungicide to the lower and underside of leaves within the soybean canopy.
[g] Fisher's protected least significant difference (LSD) was used to separate the treatment means at an alpha (α) level of 0.05.

Table 8. Treatment effect on disease control and yield across locations in Iowa in 2017 and 2018.

Small Plot			Upper canopy[a]	Middle canopy[a]		
Year	Location (Iowa)[b]	Application[c]	Frogeye severity[d]	Frogeye severity	Septoria severity[e]	Yield (kg/ha)[f]
2017	Roland	Non-treated	-	-	15.3	4297
		Traditional	-	-	18.2	4239
		Undercover	-	-	14.5	4006
		P-value[h]	-	-	0.408	0.327
2017	Story City	Non-treated	5.1	5.1	-	2682
		Traditional	3.8	3.8	-	2619
		Undercover	4.6	4.6	-	2682
		P-value	0.054	0.054	-	0.581
2018	Curtiss, Ames	Non-treated	1.9	1.4	16.3	4213
		Traditional	1.6	1.5	20.1	4244
		Undercover	1.9	1.2	18.8	4231
		P-value	0.189	0.337	0.260	0.991
2018	Hinds, Ames	Non-treated	11.5	8.7	18.1	3952
		Traditional	11.6	8.9	22.5	4248
		Undercover	12.1	8.2	18.8	3912
		P-value	0.733	0.421	0.308	0.001
2018	Kanawha	Non-treated	0.5	0.5	13.8	4347
		Traditional	0.4	0.4	6.3	4440
		Undercover	0.8	0.8	11.3	4551
		P-value	0.313	0.437	0.072	0.772
2018	Nashua	Non-treated	2.3	2.3	10.1	3898
		Traditional	1.9	1.9	6.3	4097
		Undercover	1.9	1.9	8.8	3962
		P-value	0.387	0.386	0.059	0.307
On-Farm						
2018	Nevada	Non-treated	1.9	1.7	20.1	3129
		Traditional	2.3	1.5	18.3	3152
		Undercover	1.6	1.6	18.3	3202
		P-value	0.131	0.796	0.871	0.495
2018	Roland	Non-treated	1.7	1.2	15.1	4009
		Traditional	1.3	1.2	18.3	4008
		Undercover	1.6	1.2	18.3	3853
		P-value	0.131	0.982	0.694	0.491

[a] Upper canopy was represented the top 2 to 3 nodes of the soybean plant. Middle canopy represented the bulk of the soybean plant, usually this region consisted of 10 nodes.
[b] All field experiments were conducted across Iowa. In 2018, there were two locations in Ames at separate research farmers.
[c] Non-treated = untreated control. Traditional application applied fungicide directly onto the top of a crop canopy. Undercover application applied fungicide to the top of the crop canopy as well

Table 8. (continued)

as depositing it between the rows with 3 multidirectional nozzles to increase the chance of applying a fungicide to the lower and underside of leaves within the soybean canopy.

[d] Frogeye leaf spot severity was determined estimating the percentage of the leaf area blighted.

[e] Septoria brown spot severity was determined by estimating the percentage of the leaf area blighted.

[f] Yields were adjusted to 13% seed moisture.

[g] Field trials were conducted in small plot in university research farm and replicated on-farm strip trial in farmer's field.

[h] Fisher's protected least significant difference (LSD) was used to separate the treatment means at an alpha (α) level of 0.05.

Table 9. Treatment effect on Phomopsis seed decay in Iowa in 2017 and 2018.

Year[a]	Application[b]	Seeds infected[c]
2017	Non-treated	5.2
	Traditional	4.7
	Undercover	4.9
	P-value[d]	0.513
2018	Non-treated	23.7
	Traditional	19.9
	Undercover	21.3
	P-value	0.496

[a] Each year has two small plot locations in central Iowa.
[b] Non-treated = untreated control. Traditional application applied fungicide directly onto the top of a crop canopy. Undercover application applied fungicide to the top of the crop canopy as well as depositing it between the rows with 3 multidirectional nozzles to increase the chance of applying a fungicide to the lower and underside of leaves within the soybean canopy.
[c] Number of infected seeds out of 100. Tested with blotter box.
[d] Fisher's protected least significant difference (LSD) was used to separate the treatment means at an alpha (α) level of 0.05.

Table 10. Treatment effect on Phomopsis seed decay across locations in Iowa in 2017 and 2018.

Year	Location (Iowa)[a]	Application[b]	Seeds infected[c]
2017	Roland	Non-treated	3.3
		Traditional	4.5
		Undercover	4.2
		P-value[d]	0.578
2017	Story City	Non-treated	5.5
		Traditional	4.3
		Undercover	3.3
		P-value	0.367
2018	Curtiss, Ames	Non-treated	21.2
		Traditional	19.4
		Undercover	22.4
		P-value	0.387
2018	Hinds, Ames	Non-treated	20.2
		Traditional	19.5
		Undercover	18.4
		P-value	0.418

[a] All field experiments were conducted across Iowa. In 2018, there were two locations in Ames at separate research farmers.
[b] Non-treated = untreated control. Traditional application applied fungicide directly onto the top of a crop canopy. Undercover application applied fungicide to the top of the crop canopy as well as depositing it between the rows with 3 multidirectional nozzles to increase the chance of applying a fungicide to the lower and underside of leaves within the soybean canopy.
[c] Number of infected seeds out of 100. Tested with blotter box.
[d] Fisher's protected least significant difference (LSD) was used to separate the treatment means at an alpha (α) level of 0.05.

CHAPTER 3. EFFECT OF FUNGICDE ON DURATION OF SEED FILL IN SOYBEAN

Abstract

As profit margins narrow and input costs remain high soybean farmers have had continued interest in ways to maximize soybean yield. One way to accomplish this is to use foliar fungicides to manage fungal pathogens when disease risk is high. Fungicides are also sometimes applied to increase or preserve plant health in the absence of disease. The goal of this study was to evaluate the impact of foliar fungicide on soybean seed fill in field trials. Trials were conducted in central Iowa at two locations in both 2017 and 2018. All four locations included a non-treated control and a fungicide (pyraclostrobin + fluxapyroxad) applied at the R3 growth stage. Both treatments were measured for their impact on disease control, seed growth rate, and seed filling period, and yield. Frogeye leaf spot (*Cercospora sojina*) and Septoria brown spot (*Septoria glycines*) were the two most common disease observed and severity was greater for both disease in 2018 than 2017. There were no significant difference in disease control, seed growth rate, and seed filling period between both years. Yield was 7% greater for the fungicide application at one location in 2018. The results suggest that using pyraclostrobin + fluxapyroxad was ineffective at managing foliar diseases or affecting seed fill.

Introduction

Fungicide usage in soybean has steadily increased since the early 2000s (Wise and Mueller, 2011). The increase in applications has occurred largely due to the effectiveness of fungicides to control diseases as well as being the only effective management practice for controlling soybean rust (*Phakopsora pachyrhizi*) (Schneider et al., 2005). Another reason for this increase has been apparent plant health and yield benefits of using a fungicide even in the absence of disease (Wise and Mueller, 2011; Mueller et al., 2013).

In soybean, fungicide applications purported to improve plant health have been observed as a stay-green effect (Balba, 2007; Bartlett et al., 2002). The stay-green effect refers to soybean plants whose leaves and aboveground structures remain green longer than normal (Morrison et al., 1999). It is suggested that the stay-green effect is associated with a longer seed-filling period which can contribute to greater yields (Kumudini et al., 2001; Kyverga et al., 2013).

The actual benefits of the stay-green effect, however, are controversial due to inconsistent results (Bradley and Sweets 2008; Kyverga et al., 2013). This controversy has been enhanced because the underlying physiological mechanism or developmental process causing this stay-green effect is not well understood. Early physiological studies determined that the retention of green leaf tissue was due to a hormonal shift between a senescence promoter and an inhibitor (Noodén and Leopold, 1988). Quinone outside inhibitors (QoI) are the main group of fungicides associated with improved plant health. More specifically, the group known as strobilurins have been found to alter hormones within the plant (Balba, 2007). The fungicide kresoxim-methyl derived from Strobilurin A modulates the hormonal status of the plant via a bioregulatory auxin-like activity (Grossmann et al., 1999). In wheat shoot tissue, kresoxim-methyl reduced ethylene biosynthesis and was linked to delayed leaf senescence and prolonged photosynthetic activity of the green tissue (Grossmann and Retzlaff, 1997). It is unknown if the hormone balance plays a role in prolonged soybean leaf function that could contribute to an increase in the seed filling period.

Effective filling period (EFP), phenological characterization, and estimation of the reproductive period duration using the harvest index are common ways to estimate the length of the seed filling period (Pfeiffer and Egli, 1988). The EFP can be calculated on a community or individual seed basis (Daynard et al., 1971). This method estimates the time it would take to produce yield if the seeds always grew at a linear rate. The EFP avoids the uncertainty involved with accurately estimating the beginning and end of seed growth (Egli, 1997). Seed growth rate (SGR) is determined by linear regression of dry seed weights over the period of seed development (Egli, 1997). Non-linear growth during the lag phase at the beginning of seed development and slow growth during the declining phase at the end of seed development are not considered. Removing lag phase and declining phase data from the EFP is inconsequential as most of the seed dry weight accumulates during the linear phase (Egli, 1997).

While using a fungicide for a plant health benefit may be beneficial it is important to understand the potential risk of resistance development. Fungicides, specifically the chemical compounds classified as QoIs, have a single mode of action (Mueller et al., 2013). A fungicide that acts at a single site is more likely to become less effective sooner. Resistant strains of the fungus exist naturally in a population due to naturally occurring genetic mutations. Resistant strains in a fungal population are selected for during a fungicide application since the sensitive population and only the resistant strain survives. Overtime, the population of resistant strains increase and replace the sensitive fungal population.

The goal of this research was to determine if a fungicide increased the duration or rate of soybean seed filling.

Materials and Methods

Four experiments were conducted in 2017 and 2018 in central Iowa. In 2017, plots were located near Roland, IA and Story City, IA. In 2018, plots were located at the Iowa State research farms southwest (Curtiss) and northeast (Hinds) of Ames, IA. At each location, data were collected from a larger experiment that was planted as a randomized complete block design (RCBD) with eight replications (see Chapter 2). A subset of plots from three replications were used for this experiment. Individual plots were 5.3 m long consisting of four rows spaced 76 cm apart. Seeds were sown at a rate of 309,405 seeds/ha. Weeds and insects were managed according to local recommendations (Table 1). Natural precipitation was tracked at each location (Table 2).

Two treatments were evaluated: a non-treated control and pyraclostrobin + fluxapyroxad (Priaxor, BASF, Ludwigshafen, Germany) applied at growth stage R3 (beginning pod formation) at 292 mL per hectare. At all locations, the soybean canopy was closed at the time of application. Fungicide was applied with a self-propelled plot sprayer built by Iowa State University personnel using XR11003 nozzles (TeeJet, Glendale Heights, Illinois) that traveled 6.4 km/h and applied 187 L/ha fungicide at 207 kPa.

Foliar diseases were assessed at the R6 growth stage (full seed, Fehr and Caviness, 1977) from the center rows of each small plot. Ten leaves in both the upper and middle canopy were chosen arbitrarily and frogeye leaf spot severity was visually estimated as the percent leaf area blighted. Septoria brown spot (*Septoria glycines*) severity was assessed by visually estimating the percent of leaf area covered by the disease on ten arbitrary leaves at the highest point of infection within the canopy.

Effective filling period (EFP) was calculated on an individual seed basis using the method described by Egli (1997). Calculations began at the R5 growth stage (beginning seed, Fehr and Caviness, 1977) when one soybean plant was arbitrarily sampled. Twenty seeds were collected from one plant. On average, samples were taken every five days until the R8 growth stage (full maturity). The 20 seeds were placed into 20 mL disposable scintillation vials and fresh weights were immediately taken with a portable electric balance (Scout Pro, Ohaus, Parsippany, New Jersey). Scintillation vials were dried in a Labconco FreeZone Tray Dryer (Labconco, Kansas City, MO) for 96 h at 60°C and the seeds were reweighed. To analyze on an individual seed basis, dry weight was divided by 20 (the total number of seeds).

Seed growth rate (SGR) was estimated for each plot by linear regression of dry weight (mg/seed) vs. days after first sample after all data was plotted. This estimation accounted for only samples in the linear phase and none during the beginning and end of seed growth. EFP was calculated on an individual seed basis by dividing the mature seed weight (mg) per seed by the SGR (Egli, 1997). EFP was calculated for each plot at all four locations.

Seed was mechanically harvested from the two center rows at the R8 (full maturity) growth stage (Fehr and Caviness, 1977) with a 2009 Almaco SPC20 research plot combine (ALMACO, Nevada, Iowa). Total seed weight/plot and moisture were measured. Seed weight was adjusted to 13% moisture and yield was calculated.

Data Analysis. Analysis of variance was performed using Proc GLIMMIX (version 9.4; SAS Institute Inc., Cary, North Carolina) in SAS for disease severity, seed growth rate, effective filling period, and yield. Individual locations were analyzed separately due to different soybean varieties planted at each location. The fungicide treatment was treated as a fixed factor, while replication was treated as a random factor.

Results

Frogeye leaf spot (*Cercospora sojina*) and Septoria brown spot (*Septoria glycines*) were the two most common diseases observed in all field trials and severity was greater for both diseases in 2018 than 2017 perhaps due to higher rainfall in 2018 during August and September, which are critical months for disease development. Regardless of year or location there was no significant difference in disease severity between the non-treated control and the fungicide application (Table 3).

Soybean yield differed across locations (Table 3). Yield in the non-treated control ranged from 2682 kg/ha in Story City to 4297 kg/ha in Roland in 2017. The low yield in Story City might be related to the difference in cultivar's yield potential and difference soil properties and management conditions. Regardless of the difference in yield across the locations the non-treated control and fungicide application had no significant effect on yield at three of the four locations (Table 3). There was a significant difference in yield between treatments (P=0.005, Table 3) at the Hinds Research Farm (Ames, IA) where the yield of the fungicide treatment was 7% greater than yield from non-treated plots.

Seed growth rate per day in four locations are depicted as a linear relationship in figures 1 to 4. The linear equation is derived as (y=mx+b) where the slope represents the seed growth rate and the intercept represents the seed weight at the sampling date 0. The R^2 value represents how close the seed growth rates are fitted to the regression line. R^2 values ranged from 0.86-0.97, with the majority >0.90. Sampling started after 96 to 110 days after planting at the R5 growth stage. From 96 to110 days after planting to 136 to 141 days the seed growth rate across all four locations ranged from 4.5 to 5.7 mg seed^{-1} day^{-1} (Figures 1-4). Seed growth was non-significant between treatments at all locations. The seed filling period ranged from 31 to 33 days across both years (Table 3) in both non-treated and fungicide applied plots. The duration of seed fill was a day longer in fungicide treated plots than the non-treated control in three of the four locations however the difference in duration was not statistically significant. At Hinds 2018, the fungicide treated produced 7% greater yields than the non-treated plots but, the rate of seed fill was not statistically different; 5.1 mg^{-1}day^{-1} in the non-treated plots and 4.9 mg^{-1}day^{-1} in fungicide treated plots (P=0.118).

Discussion

In our study, we were unable to demonstrate that an application of a QoI fungicide at R3 prolonged the duration of the seed filling period of soybean. The intercept of seed weight at the first sampling date 0 was greater in fungicide treated plots than the non-treated plots meaning the grain filling period may have started earlier in fungicide treated plots than the non-treated plots. The inability to see significant differences may in apart be due to sampling a new plant at each sampling date which can provide significant variability due to the timing of flowering and other environmental factors. A better technique that focuses on sampling only one seed and repeated samples from a similar position may help reduce such variability.

Pyraclostrobin has been reported to cause plant health changes in treated wheat plants such as increased leaf greenness, chlorophyll content, photosynthetic rate, water use efficiency, as well as delayed senescence (Grossmann and Retzlaff, 1997; Grossmann et al., 1999; Bryson et al., 2000). However, in other crops there have been conflicting reports that a fungicide application can result in plant health benefits (Bertelsen et al., 2001; Khan and Carlson, 2009; Nason et al., 2007; Nelson et al., 2010; Swoboda and Pedersen, 2009; Weisz et al., 2011). These inconsistent data may be conflicting due to different physiology between plant species. Additional factors could be abiotic or biotic stress, which could inflect detrimental stress that impacts yield negatively. The goal of this research was to determine if a fungicide application containing a QoI fungicide exhibited an increase in the seed filling period as a plant health benefit in soybean.

Effective filling period days fell within the standard seed growth characteristics of 22 to 33 days, respectively for soybean growth (Egli, 1997). In our study, neither seed growth rate nor duration of seed fill differed between treatments at all locations. Yield was 7% greater for the fungicide application than the non-treated control at one location (*P*=0.005). These results are similar to previous studies (Henry et al., 2011; Mahoney et al., 2015) that looked at plant health benefits associated with a fungicide application. Mahoney et al., (2015) applied pyraclostrobin to twenty soybean cultivars. Delayed maturity of twenty soybean cultivars was observed when pyraclostrobin was applied, but the impact of pyraclostrobin varied among all cultivars (Mahoney et al., 2015). Yield differences were inconsistent across years when pyraclostrobin were applied to soybean (Mahoney et al., 2015). Similarly, Henry et al., 2011 reported no significant cultivar response of a plant health benefit when pyraclostrobin applications were made to near isogenic soybean cultivars.

Seed growth rate can be influenced by other factors as well. Changes in assimilate supply can either decrease or increased the seed growth rate (Egli et al., 1985b). Reduction in the number of seeds have increased seed growth rate, while shading has decreased seed growth rate (Egli et al. 1985b). Abiotic factors like temperature can alter seed growth rate if they lower than 22 °C and higher than 30 °C (Egli and Wardlaw, 1980; Gibson and Mullins, 1996). However, abiotic factors like water stress have been shown to have little influence on seed growth rate due to mobilization of reserve assimilates, the plant reducing the number of seed produced, and maintaining seed water potential during water stressed periods (Egli, 1997; Quatter et al., 1987).

Effective filling period like seed growth rate can be impacted by numerous factors. The genotype is one of the biggest factors that impacts the seed filling period (Gay et al., 1980; Zeiher et al., 1982; Boon-Long et al., 1983). Water stress can lead to earlier maturity, a shorter grain filling period, and reduced seed size and yield (Chowdhury and Wardlaw, 1978). However, assimilate supply and temperature fluctuation between 20 °C and 30 °C do not influence the seed filling period (Hesketh et al., 1973; Egli et al., 1985a).

Another factor that may influence the duration of seed filling is the redistribution of nitrogen. Research has shown foliar fertilization of nitrogen, phosphorus, potassium, and sulfur during the seed filling period increase soybean yields (Garcia and Hanway, 1976). Applying these nutrients avoids problems of depletion of these nutrients in the leaves and the decrease in photosynthetic rate. During leaf senescence carbon and nitrogen are redistributed from the leaf to the developing seed (De Souza et al., 1997). As a result, research has shown foliar application of nitrogen can supplement the poor translocation from the leaves to the seeds and delay leaf senescence (Garcia and Hanway, 1976; De Souza et al., 1997).

The inconsistences surrounding plant health benefits suggest that the seed filling period is more strongly influenced by other abiotic and biotic factors that override a QoI fungicide's ability to increase the seed filling period. In this study, no effect of fungicide on seed fill duration was detected. Thus, either fungicides do not affect seed fill or other factors may have negated the effect of the fungicide. Drought and or heat stress may also influence the duration of seed filling period. There was no evidence of drought stress (Table 2) either year of this study, but heat and fertility stress cannot be ruled out.

It is important to point out that there were detectable levels of disease each year of this study. However, regardless of the year, no differences in fungal disease control were observed between the non-treated control and the fungicide application. The inability to see a difference in disease control suggests foliar pathogen populations have become resistant to QoI fungicides in Iowa (Zhang et al., 2018; Mueller et al., 2019; Neves et al., 2019).

These results support some previous studies that evaluated the economics of using a QoI fungicide as a means to improve plant health (Swoboda and Pedersen, 2009; Henry et al., 2011). Swoboda and Pedersen (2009) suggested there was a low probability that a fungicide application increased soybean yield by mechanisms other than disease control. The combination of increased application costs and the inability to control foliar soybean pathogens emphasizes that fungicides should be used responsibly for their intended purpose of fungal disease management in soybean.

Conclusion Fungicide application did not influence on the duration or rate of seed filling. Yield benefit to fungicide was observed only in one of the four locations. The combination of increased cost with the inability to control foliar soybean pathogens emphasizes the practice that fungicides should be used responsibly for their intended purpose of fungal disease management in soybean. Fungal resistance may have, at least in part, influenced our ability to determine differences in disease control, seed growth rate, duration of seed filling, and ultimately yield.

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Figures

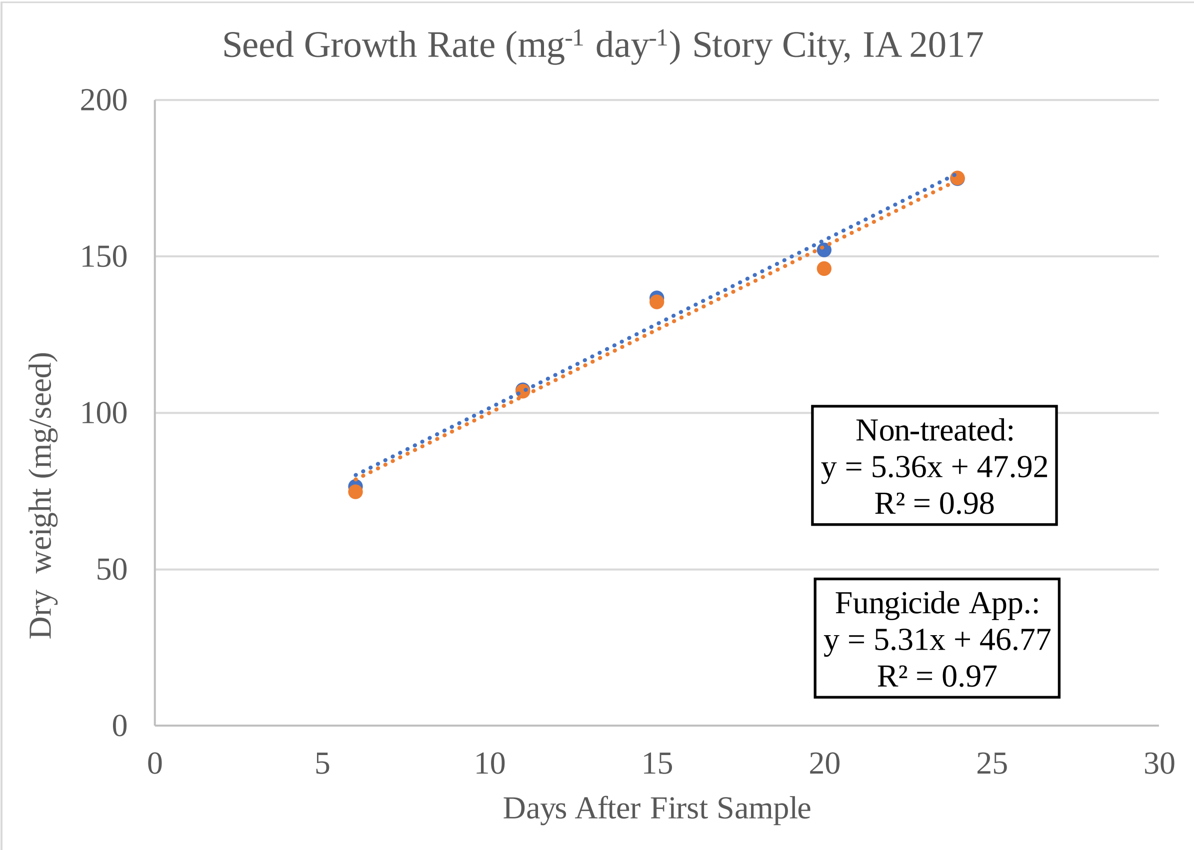

Figure 1. Seed growth rate in the linear phase for soybean either not treated (blue) or treated with pyraclostrobin + fluxapyroxad (orange) in Story City, IA 2017.

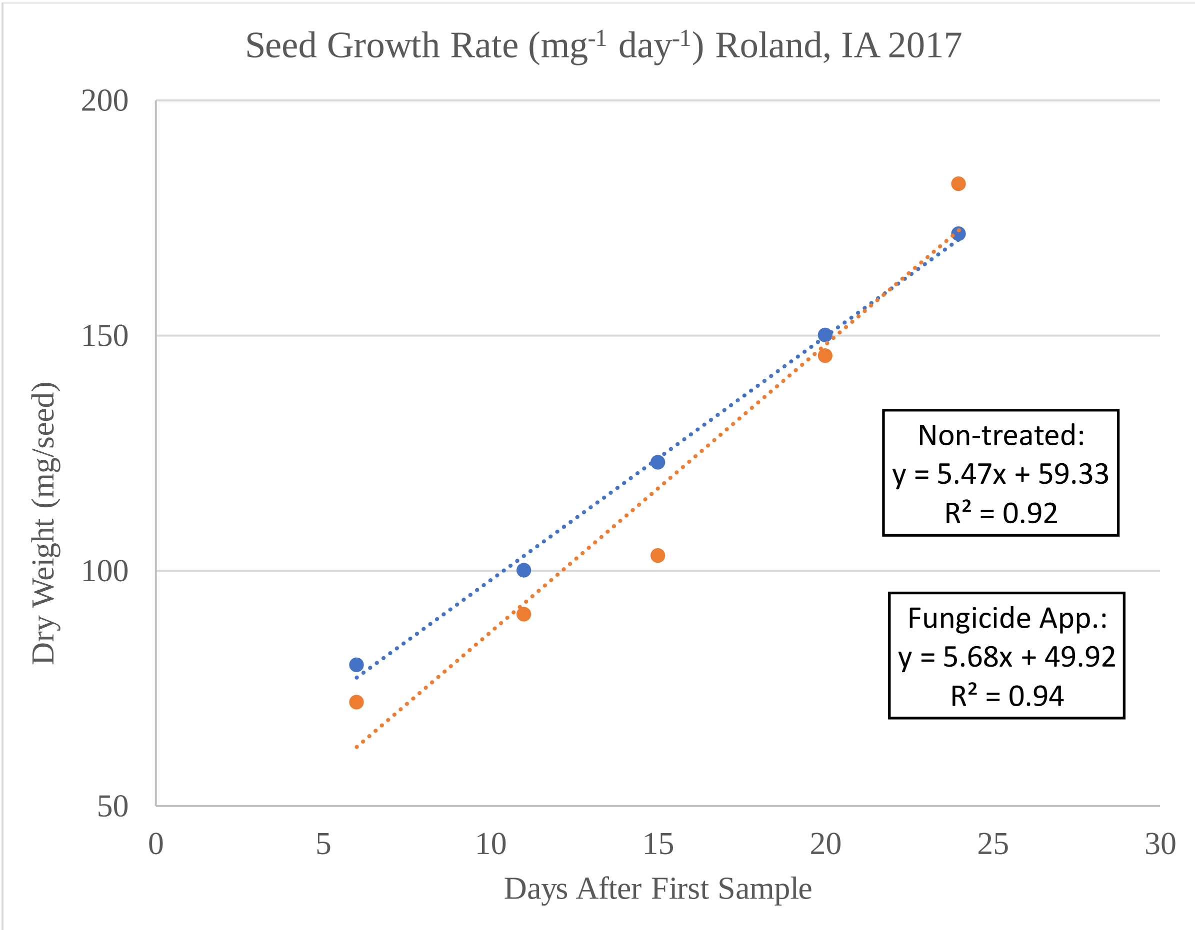

Figure 2. Seed growth rate in the linear phase for soybean either not treated (blue) or treated with pyraclostrobin + fluxapyroxad (orange) in Roland, IA 2017.

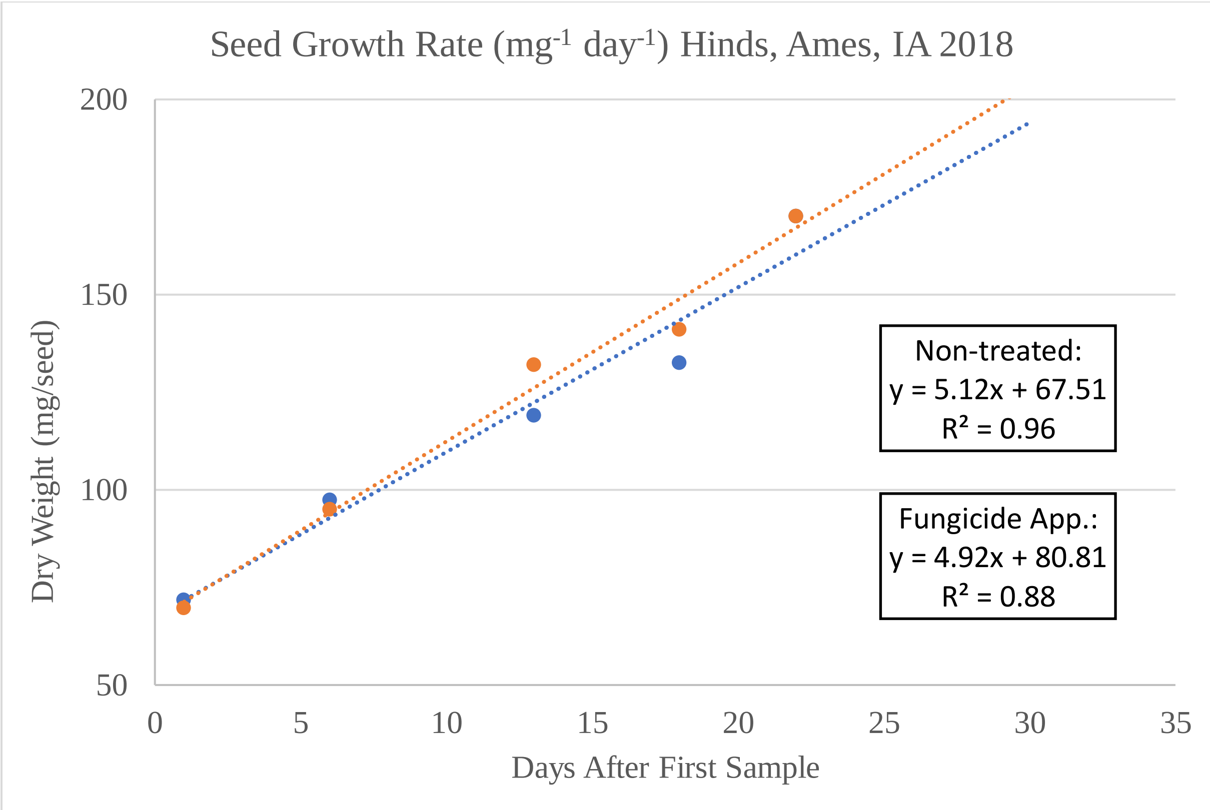

Figure 3. Seed growth rate in the linear phase for soybean either not treated (blue) or treated with pyraclostrobin + fluxapyroxad (orange) in Hinds, Ames, IA 2018.

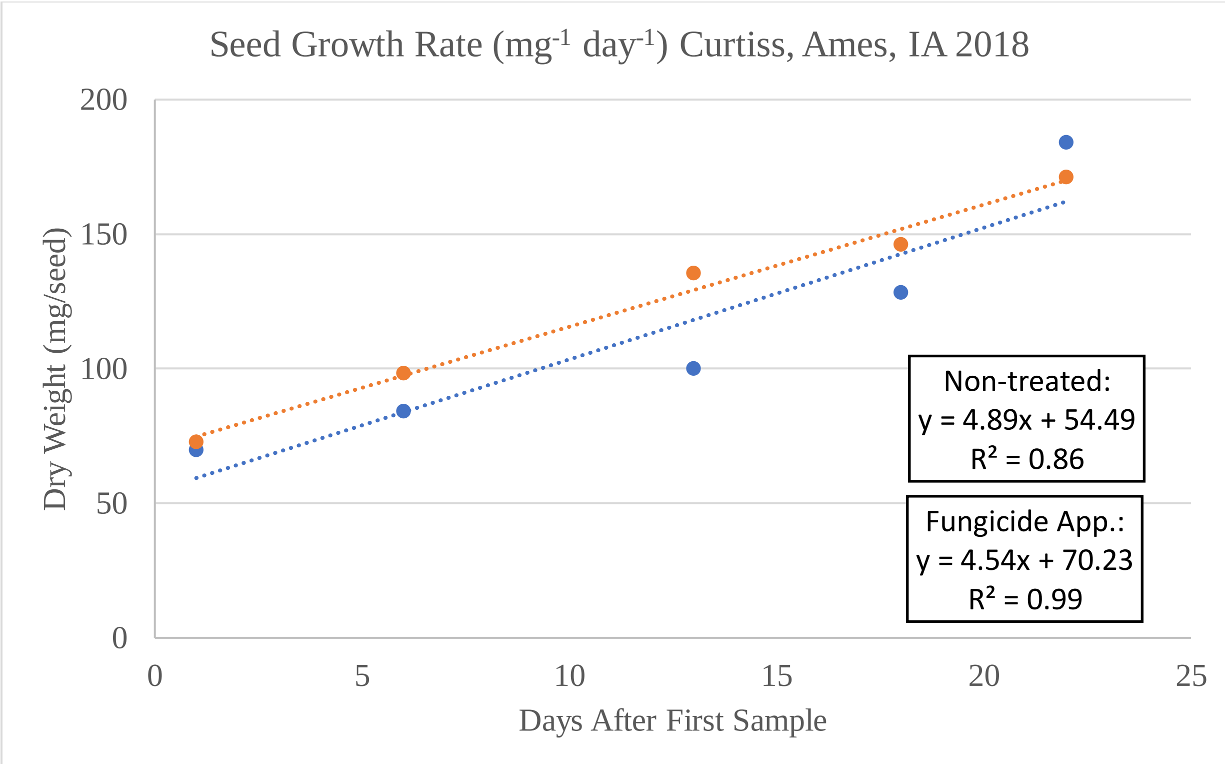

Figure 4. Seed growth rate in the linear phase for soybean either not treated (blue) or treated with pyraclostrobin + fluxapyroxad (orange) in Curtiss, Ames, IA 2018.

Tables

Table 1. Site information for trials examining the impact of fungicide on the seed filling period of soybean in Iowa in 2017 and 2018.

Year	Location (Iowa)[b]	GPS Coordinates	Variety	Planted population (seeds/ha)	Herbicide[a]	
					Preemergence	Postemergence
2017	Roland	42.123552, -93.500943	AG23x8	309,405	pyroxasulfone, metribuzin,	glyphosate
2017	Story City	42.202926, -93.541542	AG2535	334,158	pendimethalin, pyroxasulfone	glyphosate, imazamox
2018	Curtiss, Ames	42.003574, -93.662752	S28C6	309,405	saflufenacil	glyphosate
2018	Hinds, Ames	42.061962, -93.617633	AG24x7	309,405	pendimethalin	glyphosate

[a] Roland, IA 2017 – (Zidua, 2.0 oz./acre, BASF), (Sencor 75DF, 150 g/acre, Bayer Environmental Science) preemergence, (RoundUp PowerMAX, 48 oz./acre, Bayer Environmental Science) postemergence. Story city, IA 2017 – (Prowl H2O, 24 oz./acre, BASF), (Zidua PRO, 4.5 fl. oz./acre, BASF) preemergence, (Touchdown total 64 fl. oz./acre, Syngenta), (Raptor, 4 fl. oz./acre, BASF) postemergence. Curtiss farm, IA 2018 – (Sharpen, 1.0 fl.oz./acre, BASF) preemergence, (RoundUp WeatherMAX, 42 fl.oz./acre, Bayer Environmental Science) postemergence. Hinds farm, IA 2018 – (Prowl H2O, 48 oz./acre, BASF) preemergence, (RoundUp PowerMAX 40 fl.oz./acre, Bayer Environmental Science) postemergence.
[b] All field experiments were conducted in central Iowa. In 2018, there were two locations in Ames at separate research farmer.

Table 2. Monthly precipitation recorded with Iowa Environmental Mesonet during the soybean growing season in central Iowa in 2017 and 2018.

		Monthly precipitation (cm)						
Year	Location (Iowa)	May	June	July	August	September	October	Total
2017	Roland	10.1	4.3	3.6	5.1	5.1	6.5	34.7
2017	Story City	12.2	5.9	3.6	5.4	4.3	7.3	38.7
2018	Curtiss, Ames	8.7	2.9	4.1	6.4	8.6	12.4	43.1
2018	Hinds, Ames	10.1	3.8	3.2	5.7	9.6	11.4	43.8

Table 3. Effect of fungicide application on disease control, seed filling period, and yield in soybean from trials in central Iowa in 2017 and 2018.

	2017		2018	
	Frogeye leaf spot severity[a]			
	Story City	Roland	Hinds, Ames	Curtiss, Ames
Non-treated	5.6	.	10.2	2.1
Fungicide App.	4.1	.	11.5	1.9
P-value	0.191	.	0.554	0.281
	Septoria brown spot severity[b]			
	Story City	Roland	Hinds, Ames	Curtiss, Ames
Non-treated	.	15.2	14.3	16.5
Fungicide App.	.	14.1	13.2	18.4
P-value	.	0.711	0.496	0.265
	Seed filling period (days)[c]			
Non-treated	32	31	32	33
Fungicide App.	33	32	33	33
P-value	0.423	0.184	0.118	0.556
	Yield (kg/ha)[d]			
Non-treated	2682	4297	3952	4213
Fungicide App.	2619	4239	4248	4244
P-value	0.155	0.241	0.005	0.137

[a] Frogeye leaf spot severity was determined estimating the percentage of the leaf area diseased.
[b] Septoria brown spot severity was determined by selecting the uppermost leaf infected with disease and estimating the percentage of the leaf area diseased.
[c] Seed filling was calculated by dividing the final seed weight by the rate of accumulation of dry matter by the seed during the linear phase of seed growth.
[d] Total seed weight and moisture were measured. Seed weight was adjusted to 13 percent moisture and yield was calculated.

Supplemental Tables.

Supplemental Table 1. Dry seed weight collected from the R5-R8 growth stages in 2017 and 2018 in Iowa.

Story City, 2017

	Days After First Sample								
	0	6	11	15	20	24	28	31	.
	Days After Planting								
	105	111	116	120	125	129	133	136	.
	Growth Stage								
	R5	R5	R6	R6	R7	R7	R8	R8	
Treatment	Dry Weight (mg/seed)								
Non-treated	73a	81	121	131	143	180	179	178	.
Non-treated	68	74	85	142	166	175	175	175	.
Non-treated	71	76	85	138	155	170	169	170	.
Fungicide	69	73	117	136	146	175	175	173	.
Fungicide	74	76	114	134	143	180	175	175	.
Fungicide	68	76	90	138	151	177	175	175	.

Roland 2017

	Days After First Sample								
	0	6	11	15	20	24	28	31	.
	Days After Planting								
	110	116	121	125	130	134	138	141	
	Growth Stage								
	R5	R5	R6	R6	R7	R7	R8	R8	
Treatment	Dry Weight (mg/seed)								
Non-treated	41	90	129	136	132	175	174	175	.
Non-treated	40	78	112	112	113	170	170	169	.
Non-treated	46	73	118	121	123	170	169	170	.
Fungicide	38	66	91	109	159	187	185	186	.
Fungicide	42	74	92	103	138	183	180	182	.
Fungicide	43	76	90	102	141	178	177	178	.

Supplemental Table 1. (continued)

Hinds, Ames 2018

	Days After First Sample								
	0	6	13	18	22	26	30	36	40
	Days After Planting								
	101	107	114	119	123	127	131	137	141
	Growth Stage								
	R5	R5	R6	R7	R7	R8	R8	R8	R8
Treatment	Dry Weight (mg/seed)								
Non-treated	78	112	124	145	153	155	159	159	159
Non-treated	83	86	110	128	135	150	163	163	163
Non-treated	81	95	124	125	153	155	177	175	177
Fungicide	81	111	138	158	160	160	169	168	168
Fungicide	65	105	146	152	156	153	168	168	167
Fungicide	64	102	128	157	164	179	178	179	179

Curtiss, Ames, 2018

	Days After First Sample								
	0	6	13	18	22	26	30	36	40
	Days After Planting								
	96	102	109	114	118	122	126	132	136
	Growth Stage								
	R5	R5	R6	R7	R7	R8	R8	R8	R8
Treatment	Dry Weight (mg/seed)								
Non-treated	85	115	125	131	151	160	159	160	160
Non-treated	60	80	101	118	136	159	160	159	160
Non-treated	69	83	121	136	155	157	159	160	159
Fungicide	97	115	127	144	155	156	163	160	160
Fungicide	72	110	142	146	144	154	161	160	159
Fungicide	60	70	137	149	149	150	155	160	158

[a] Seed weight is the average of twenty seeds collected.

CHAPTER 4. SUMMARY

General Conclusion

Soybean foliar fungicides are traditionally applied over the top of the plant canopy to manage disease. In an attempt to distribute fungicide throughout the soybean canopy and achieve improved disease control, undercover spray applications were developed to place fungicides within the soybean canopy using multidirectional nozzles as well as nozzles above the canopy. In an effort to determine if a difference existed between traditional and undercover spray application methods two years of research were conducted at multiple field locations.

It was found that both traditional and undercover fungicide application methods provided coverage to the upper, middle, and lower soybean canopy as determined by both spray cards and tracer dye detection methods. Each year during this research, frogeye leaf spot and Septoria brown spot were present at adequate levels. However, no significant differences in disease control were observed between traditional and undercover fungicide application treatments, nor was there a significant yield difference between treatments.

Research on fungicide application to improve plant health was ineffective. There were no significant differences between fungicide application and the non-treated control for seed growth rate and the duration of seed filling. At three of the four locations, the difference in yield was non-significant between the treatments. The combination of increased production costs with the inability to control foliar soybean pathogens emphasizes the practice that fungicides should be used responsibly to mitigate the development of fungal resistance. Fungal resistance may have, at least in part, influenced our ability to determine differences in disease control, seed growth rate, duration of seed filling, and ultimately yield.

Soybean farmers should be aware that fungicide resistance for the pathogens that cause frogeye leaf spot and Septoria brown spot have been identified in Iowa. Resistance to these two pathogens made it difficult to determine which fungicide application method was more effective. Future work comparing the traditional versus undercover application may be necessary with a more effective fungicide.

APPENDIX. WHITE MOLD

White mold (*Sclerotinia sclerotiorum*) can cause significant yield loss, especially during cool, wet years (Adams and Ayers 1979, Grau and Hartman 1999, Allen et al., 2017). Practices like tillage, early planting, narrow row widths, and higher plant populations favor disease development (Wu and Subbarao 2008). Chemical control is one management tool, but can be challenging due to disease development deeper in a plant canopy. Furthermore, chemical control can be challenging when using a spray that applies the chemical product from above the plant canopy. As a result, technology, like the undercover application that runs between the rows with three multi-directional nozzles, may improve fungicide efficacy for diseases such as white mold.

In 2018, two small plot experiments were conducted in Kanawha and Nashua, Iowa. Each location was organized as a randomized complete block design with four replications. All locations included three treatments: (1) non-treated control, (2) fungicide applied using a traditional sprayer, and (3) fungicide applied using an undercover sprayer. Each plot replication consisted of four rows (6.1 m long with 76.2 cm row-row spacing). Endura (boscalid, BASF, Ludwigshafen, Germany) was applied at 350 g/hectare. Applications were made at a speed of 6.4 km/h, with 207 kPa to deliver 187 L/ha.

Disease incidence was inadequate for determining differences between treatments. Future investigation of the traditional and undercover application methods in years with more disease pressure could help determine if improving coverage with undercover applications can better manage white mold.

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Tables

Table 1. Effect of application method on white mold in soybean in Kanawha and Nashua, Iowa in 2018.

	Disease Incidence[a]	Severity[a]	Disease Severity Index[a]	Yield (kg/ha)[b]
Kanawha				
Non-treated[c]	2.5	1.2	2.2	4573
Traditional	0.83	0.5	0.6	4708
Undercover	2.5	1.3	1.9	4775
P-value[d]	0.729	0.723	0.645	0.785
Nashua				
Non-treated	0	0	0	3968
Traditional	0.8	0.3	0.6	4035
Undercover	0.8	0.4	0.8	4035
P-value	0.636	0.614	0.614	0.336

[a] White mold severity: 0-3 scale, where 0 = no infection, 1 = infection only on branches, 2 = infection on the main stem but pod fill was normal, and 3 = infection on the main stem resulting in plant death and poor pod fill. Plants were inspected in 20 random spots in the center of each plot. The 20 scores were totaled and divided by 60 (the total if all 20 scores were given a rating of 3) and multiplied by 100 to give a disease severity index (DSI).
[b] Yields were adjusted to 13% seed moisture
[c] Non-treated = untreated control. Traditional application applied fungicide directly onto the top of a crop canopy. Undercover application applied fungicide to the top of the crop canopy as well as depositing fungicide between the rows with 3 multidirectional nozzles to increase the chance of applying a fungicide to the lower and underside of leaves within the soybean canopy.
[d] Fisher's protected least significant difference (LSD) was used to separate the treatment means at an alpha (α) level of 0.05.